

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

THIS PAGE BLANK (USPTO)



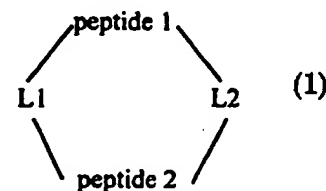
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/78, 7/56, A61K 38/39, 38/12		A1	(11) International Publication Number: WO 97/02289
			(43) International Publication Date: 23 January 1997 (23.01.97)
(21) International Application Number: PCT/GB96/01580 (22) International Filing Date: 2 July 1996 (02.07.96) (30) Priority Data: 9513798.0 6 July 1995 (06.07.95) GB 9611470.7 1 June 1996 (01.06.96) GB (71) Applicant (for all designated States except US): ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): DUTTA, Anand, Swaroop [GB/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). (74) Agent: BRYANT, Tracey; Zeneca Pharmaceuticals, Intellectual Property Dept., Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report.	

(54) Title: PEPTIDE INHIBITORS OF FIBRONECTINE

(57) Abstract

Cyclic dimeric peptides of formula (1) wherein: peptide 1 and peptide 2 independently represent a tetrapeptide of formula -AA1-AA2-AA3-AA4- juxtaposed in parallel or antiparallel orientation; AA1 is an L or D amino acid selected from Ile, Leu and amino analogues thereof selected from Pro, Gly, Tic and Phe; AA2 is an L amino acid selected from Leu and amino acid analogues thereof selected from Ile, Phe and Val; AA3 is an L amino acid selected from Asp, Glu and amino acid analogues thereof; AA4 is an L amino acid selected from Val and amino acid analogues thereof selected from Leu, Ile, Phe and Cha (cyclohexylalanine); L1 and L2 independently represent linking moieties for linking peptides 1 and 2 to form a cyclic dipeptide; or salts thereof. The cyclic dipeptides inhibit the interaction of vascular cell adhesion molecule-1 and fibronectin with integrin very late antigen 4 and have therapeutic applications such as in rheumatoid arthritis, asthma or multiple sclerosis.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

PEPTIDE INHIBITORS OF FIBRONECTINE

Many of the cell-cell and cell-extracellular matrix interactions are mediated by protein ligands (e.g. fibronectin, vitronectin and VCAM-1) and their integrin receptors [e.g. VLA-4 ($\alpha 4 \beta 1$)]. Recent studies have shown these interactions to play an important role in many physiological (e.g. embryonic development and wound healing) and pathological (e.g. tumour-cell invasion and metastasis, inflammation, atherosclerosis and autoimmune diseases) conditions. Agents which can selectively inhibit some of these interactions are predictably useful for the treatment of a number of diseases.

Integrins are heterodimeric cell surface receptors that are composed of noncovalently associated α and β subunits. Using molecular biology and protein chemistry, a number of α and β subunits have been identified. The integrin family can be subdivided into classes based on the β subunits, which can be associated with one or more α subunits. The most widely distributed integrins belong to the $\beta 1$ class, also known as the very late antigens (VLA). The second class of integrins are leukocyte-specific receptors and consist of one of three α subunits (αL , αM , or αX) complexed with the $\beta 2$ protein. The cytoadhesins $\alpha I I b \beta 3$ and $\alpha v \beta 3$, constitute the third class of integrins.

A wide variety of proteins serve as ligands for integrin receptors. In general, the proteins recognised by integrins fall into one of three classes: extracellular matrix proteins, plasma proteins, and cell surface molecules. Extracellular matrix proteins such as collagen, fibronectin, fibrinogen, laminin, thrombospondin, and vitronectin bind to a number of integrins. Many of these adhesive proteins also circulate in plasma and bind to activated blood cells. Additional components in plasma that are ligands for integrins include fibrinogen and factor X. Cell-bound complement C3bi and several transmembrane proteins, such as Ig-like cell adhesion molecule (ICAM-1,2,3) and vascular cell adhesion molecule (VCAM-1), which are members of the Ig superfamily, also serve as cell-surface ligands for some integrins.

The target amino acid sequences for many integrins have been identified. For example, the target sequence in $\alpha 5 \beta 1$, $\alpha I I \beta 3$, and $\alpha v \beta 3$, is the Arg-Gly-Asp tripeptide found in proteins such as fibronectin, fibrinogen, thrombospondin, type 1 collagen, vitronectin and vWF. However, the Arg-Gly-Asp sequence is not the only integrin

recognition motif used by adhesive ligands. Another integrin $\alpha 4 \beta 1$ binds the variable region (CS1) of fibronectin via the sequence Leu-Asp-Val and the platelet integrin $\alpha \text{IIb} \beta 3$ also recognises the sequence His-His-Leu-Gly-Gly-Ala-Lys-Gln-Ala-Gly-Asp-Val at the carboxy-terminus of the gamma chain of fibrinogen.

5 The present invention principally relates to agents which block the interaction of the ligand VCAM-1 to its integrin receptor VLA-4 ($\alpha 4 \beta 1$). [Reference for a review on VLA-4: Structure of the Integrin VLA-4 and Its Cell-Cell and Cell Matrix Adhesion Functions, M.E. Hemler, M.J. Elices, C. Parker and Y. Takada, Immunological Reviews, 114 (1990) 45-65.] Integrin $\alpha 4 \beta 1$ is expressed on numerous hematopoietic cells and
10 established cell lines, including hematopoietic precursors, peripheral and cytotoxic T lymphocytes, B lymphocytes, monocytes, thymocytes and eosinophils. Unlike other $\beta 1$ integrins that are involved only in cell-extracellular matrix interactions, $\alpha 4 \beta 1$ mediates both cell-cell and cell-extracellular matrix interactions. Cells expressing activated $\alpha 4 \beta 1$ bind to the carboxy-terminal cell binding domain of fibronectin (non Arg-Gly-Asp
15 mediated), to VCAM-1 expressed on endothelial cells, and to each other to promote homotypic aggregation. The expression of VCAM-1 by endothelial cells is upregulated by proinflammatory cytokines such as $\text{INF-}\gamma$, $\text{TNF-}\alpha$ and $\text{IL-1}\beta$.

Regulation of $\alpha 4 \beta 1$ -mediated cell adhesion is important in numerous physiologic processes, including T-cell proliferation, B-cell localisation to germinal
20 centres, and adhesion of activated T cells and eosinophils to endothelial cells. In addition, integrin $\alpha 4 \beta 1$ -mediated processes are implicated in several diseases such as melanoma cell invasion in metastasis, T-cell infiltration of synovial membranes in rheumatoid arthritis, autoimmune diabetes, colitis and leukocyte penetration of the blood-brain barrier in experimental autoimmune encephalomyelitis, atherosclerosis, peripheral vascular disease,
25 cardiovascular disease and multiple sclerosis. Evidence for the involvement of VLA-4/VCAM-1 interaction in the above disease processes has been accumulated by investigating the role of the peptide CS-1 and antibodies specific for VLA-4 or VCAM-1 in various in vitro and in vivo experimental models of inflammation (e.g. contact cutaneous hypersensitivity response in mice), experimental autoimmune encephalomyelitis, lung
30 antigen challenge, diabetes, ulcerative colitis, nephritis and allograft rejection. Further

relevant diseases include asthma, psoriasis, restenosis, myocarditis and inflammatory bowel disease.

For example, in an experimental model of arthritis (arthritis induced in inbred female Lewis rats with a single intraperitoneal injection of peptidoglycan-polysaccharide fragments from group A streptococcal cell walls), intravenous administration of CS-1 at the initiation of arthritis (days 0-4; 300 µg/day) or on days 11 to 16 in animals with established arthritis, was shown to suppress both acute and chronic inflammation. [Reference : Synthetic Fibronectin Peptides Suppress Arthritis in Rats by Interrupting Leukocyte Adhesion and Recruitment, S.M. Wahl, J.B. Allen, K.L. Hines, T. Imamichi, A.M. Wahl, L.T. Furcht and J.B. McCarthy, J. Clin. Invest., 94 (1994) 655-662].

In another model of inflammation (contact hypersensitivity response in oxazalone or 2,4-dinitrofluorobenzene-sensitised mice), intravenous administration of the anti- α -4 specific monoclonal antibodies R1-2 or PS/2 (4 to 6 hours prior to challenge) significantly inhibited (50-60% reduction in the ear swelling response) the efferent response. [Reference: Monoclonal Antibodies to the Integrin α -4 Subunit Inhibit the Murine Contact Hypersensitivity Response, P.L. Chisholm, C.A. Williams and R.R. Lobb, Eur. J. Immunol., 23 (1993) 682-688]. In an intestinal inflammation model (acute colitis in Cotton-top tamarin), anti- α 4 integrin monoclonal antibody HP1/2 that binds VLA-4 resulted in significant attenuation of acute colitis. In contrast, two anti-E-selectin monoclonal antibodies (BB11 and EH8) slightly diminished colitis after the 10-day treatment period in Cotton-top tamarin [Reference: Attenuation of Colitis in the Cotton-top Tamarin by Anti- α 4 Integrin Monoclonal Antibody, D.K. Podolsky, R. Lobb, N. King, C.D. Benjamin, B. Pepinsky, P. Sehgal and M. deBeaumont, J. Clin. Invest., 92 (1993) 372-380].

The antibodies have also been shown to be effective in a model of autoimmune encephalomyelitis (EAE), an inflammatory condition of the central nervous system with similarities to multiple sclerosis. In both diseases, circulating leukocytes penetrate the blood-brain barrier and damage myelin, resulting in impaired nerve conduction and paralysis. EAE can be induced actively by priming an animal to CNS proteins like myelin basic protein (MBP), or adoptively by injection of activated lymphocytes that are specific for these CNS antigens. Various monoclonal antibodies.

[MK/1 (anti-VCAM-1) and PS/2 and LPAM-1 (anti $\alpha 4$ integrin)], when injected into irradiated female (PL/J x SJL)F1 mice delayed the onset of disease. When injection of antibody to $\alpha 4$ integrin (LPAM-1 and PS/2) was continued every 3 day until after onset of disease, not only was the onset of disease delayed, but in this case severity of disease was also significantly decreased. [Reference: Surface Expression of $\alpha 4$ Integrin by CD4 T Cells Is Required for Their Entry into Brain Parenchyma, J.L. Baron, J.A. Madri, N.H. Ruddle, J. Hashim and C.A. Janeway, Jr., J. Exp. Med., 177 (1993) 57-68].

Antibodies specific for both $\alpha 4$ -integrin (LPAM-1) and one of its ligands, VCAM-1, were also shown to be effective in treating insulin-dependent diabetes mellitus in the nonobese diabetic mouse. Insulin-dependent diabetes mellitus is believed to be an autoimmune disease in which activated T lymphocytes destroy the insulin-producing β -cells of the pancreatic islets. The antibody R1-2 prevented the onset of insulinitis in a dose-dependent manner in nonobese diabetic mice. The blocking of disease was accompanied by a marked decrease in lymphocytic infiltration of the islets of Langerhans. [Reference: The Pathogenesis of Adoptive Murine Autoimmune Diabetes Requires an Interaction Between $\alpha 4$ -Integrins and Vascular Cell Adhesion Molecule-1, J.L. Baron, E-P. Reich, I. Visintin and C.A. Janeway, Jr., J. Clin. Invest., 93 (1994) 1700-1708].

Cells expressing integrin $\alpha 4\beta 1$ have been shown to bind to sequences in the heparin II binding domain and the alternatively spliced type III connecting segment (IIICS) located in the carboxy-terminal cell binding domain of fibronectin. Within the IIICS region, $\alpha 4\beta 1$ binds with high affinity to a peptide sequence termed CS-1 (a 25-amino acid peptide), suggesting that this is the major site of $\alpha 4\beta 1$ interaction in fibronectin. The tripeptide Leu-Asp-Val is the minimal sequence within CS-1 capable of supporting hematopoietic cell adhesion or of inhibiting $\alpha 4\beta 1$ -mediated cell binding to fibronectin. [References for CS1: The Minimal Essential Sequence for a Major Cell Type-Specific Adhesion Site (CS1) Within the Alternatively Spliced Type III Connecting Segment Domain of Fibronectin is Leucine-Aspartic Acid-Valine, A. Komoriya, L.J. Green, M. Mervic, S.S. Yamada, K.M. Yamada and M.J. Humphries, J. Biol. Chem., 266 (1991) 15075-15079; Activation-Dependent Recognition by Hematopoietic Cells of the LDV Sequence in the V Region of Fibronectin. E.A. Wayner and N.L. Kovach, J. Cell Biol., 116 (1992) 489-497.].

- 5 -

In addition to the Leu-Asp-Val containing sequences mentioned above, a cyclic octapeptide 1-adamantaneacetyl-Cys-Gly-Arg-Gly-Asp-Ser-Pro-Cys (containing a disulphide bridge between the two cysteine residues) has been reported to be as effective as the LDV containing peptide Cys-Leu-His-Gly-Pro-Glu-Ile-Leu-Asp-Val-Pro-Ser-Thr in blocking Jurkat cell adhesion to CS-1 coated plates (IC_{50} 30 μ M). The cyclic peptide also inhibited the binding of Jurkat cells to fibronectin coated plates. In addition to inhibiting $\alpha 4\beta 1$ -induced adhesion, the octapeptide also inhibited function in $\alpha v\beta 3$ as well as $\alpha IIb\beta IIIa$ -dependent assays. Therefore the peptide is not selective for $\alpha 4\beta 1$ -mediated adhesion. [Reference: Cyclic RGD Peptide Inhibits $\alpha 4\beta 1$ Interaction with Connecting Fragment 1 and Vascular Cell Adhesion Molecule, P.M. Cardarelli, R.R. Cobb, D.M. Nowlin, W. Scholz, F. Gorcsan, M. Moscinski, M. Yasuhara, S-L. Chiang and T.J. Lobl, J. Biol. Chem., 269 (1994) 18668-18673.]

A few small peptides [Reference: Non-peptidic Surrogates of the Leu-Asp-Val Sequence and Their Use in the Treatment of Inflammation, Autoimmune Diseases and Tumour progression, YEDA Research and Development Co. Ltd, WO 94/02445, Publ. date 3 Feb. 1994] have also been reported to inhibit $\alpha 4\beta 1$ -induced adhesion.

A disulphide cyclic pentapeptide, Arg-Cys-Asp-thioprolin-Cys (thioprolin = thiazolidine-4-carboxylic acid), has also been reported to be an inhibitor of leukocyte cell adhesion to fibronectin. In addition, the cyclic peptide also inhibited the binding to the 120 kDa chymotryptic fragment of fibronectin, which contains the Arg-Gly-Asp central cell binding domain. Again, the peptide was not selective. It binds to both $\alpha 4\beta 1$ and $\alpha 5\beta 1$ [Reference: A Novel Cyclic Pentapeptide Inhibits $\alpha 4\beta 1$ and $\alpha 5\beta 1$ Integrin-Mediated Cell Adhesion, D.M. Nowlin, F. Gorcsan, M. Moscinski, S-L. Chiang, T.J. Lobl and P.M. Cardarelli, J. Biol. Chem., 268 (1993) 20352-20359.]

The present invention is based on the discovery that relatively small dicyclic peptides can potently inhibit the interaction of VCAM-1 with integrin VLA4.

According to one aspect of the present invention there is provided a cyclic dimeric peptide of formula 1 (figure 1) wherein:

peptide 1 and peptide 2 independently represent a tetrapeptide of formula -AA1-AA2-AA3-AA4- juxtaposed in parallel or antiparallel orientation;

- 6 -

and

AA1 is an L or D amino acid selected from Ile, Leu and amino analogues thereof selected from Pro, Gly, Tic, Gln and Phe;

AA2 is an L amino acid selected from Leu and amino acid analogues thereof selected from

5 Ile, Phe, Pro and Val;

AA3 is an L amino acid selected from Asp, Glu and amino acid analogues thereof;

AA4 is an L amino acid selected from Val and amino acid analogues thereof selected from Leu, Ile, Phe, Ser, Pro and Cha (cyclohexylalanine);

and

10 L1 and L2 independently represent linking moieties for linking peptides 1 and 2 to form a cyclic peptide; or salt thereof.

The present invention also provides a cyclic dimeric peptide of formula 1 (Figure 1) wherein:

peptide 1 and peptide 2 independently represent a tetrapeptide of formula

15 -AA1-AA2-AA3-AA4- juxtaposed in parallel or antiparallel orientation and

AA1 represents an L or D amino acid selected from Ile, Leu, Pro, Gly, Tic, Phe and amino acid analogues thereof

AA2 represents an L amino acid selected from Leu, Ile, Phe, Val and amino acid analogues thereof

20 AA3 represents an L amino acid selected from Asp, Glu and amino acid analogue thereof

AA4 represents an L amino acid selected from Val, Leu, Ile, Phe, Cha (cyclohexylalanine) and amino acid analogues thereof and

L1 and L2 independently represent linking moieties for linking peptides 1 and 2 to form a cyclic peptide;

25 or a salt thereof.

In this specification: the tetrapeptide -AA1-AA2-AA3-AA4- has its N-terminus at AA1 and its C-terminus at AA4 unless otherwise stated; and amino acids have L configuration unless otherwise stated.

The cyclic peptide preferably has an IC₅₀ of <20μM, more preferably <15μM,
30 in the MOLT-4 cell/fibronectin assay described herein or the peptide having an IC₅₀ of

<100 μ M, preferably <50 μ M, in the MOLT-4 cell/recombinant soluble VCAM-1 assay described herein.

Preferably AA1-4 have the general formula 4 (Figure 4) wherein R¹ is the amino acid side chain and R² and R³ independently represent H or C₁₋₄alkyl (preferably H or Me, especially H).

When peptides 1 & 2 are in antiparallel juxtaposition L1 and L2 preferably have the general Formula 5 (Figure 4) in which R⁴ represents H or C₁₋₄alkyl (especially H), and X is selected from -(CH₂)_n wherein n= 1-4 optionally substituted on CH₂ with C₁₋₄alkyl; and -(CH₂)_n wherein n=1-4 optionally substituted on -CH₂ with NH₂; or
 10 X and NR⁴ together represent a group of Formula 8. Formula 8a or Formula 9 (Figure 4) in which n= 0-4.

When peptides 1 & 2 are in parallel juxtaposition L1 and L2 preferably have the general Formulas 7 and 6 (Figure 4) respectively in which R⁵ and R⁶ independently represent H or C₁₋₄alkyl (especially H), X represents -(CH₂)_n- wherein n= 1-4 optionally
 15 substituted on CH₂ with C₁₋₄alkyl or;
 X and NR⁵ together represent a group of Formula 8, Formula 8a or Formula 9 (Figure 4) in which n= 0-4 or;
 X and NR⁶ together represent a group of Formula 8, Formula 8a or Formula 9 (Figure 4) in which n= 0-4;
 20 provided that if both X & NR⁵ and X & NR⁶ represent any combination of formulas 8, 8a or 9 then the -(CH₂)_n- motif is not duplicated.

Preferred values are:

- AA1 is Ile
- 25 AA2 is Leu
- AA3 is Asp
- AA4 is Val

Preferred Ile analogues are shown in Figure 8.

Preferably peptides 1 & 2 are in antiparallel juxtaposition.

- 30 Preferably L1 and L2 are identical.

Suitable values for AA1 include tert-Leu and tert-butyl-Ala. Further suitable values for AA1 include D-Leu, MeIle and MePhe. A further suitable value for AA2 is Nle, and for AA4 Leu, Nva or Nle.

Preferred values for L1 and L2 are shown in Figure 7 for when peptides 1 & 2 are in
5 antiparallel juxtaposition. Preferred values for L1 and L2 are shown in Figure 12 for when peptides 1 & 2 are in parallel juxtaposition. Further preferred values for L1 and L2 are shown in Figure 17.

The cyclic dipeptides of the present invention have at least one of the following advantages: they are more potent than known compounds, e.g. CS1 and the
10 compound claimed in the YEDA patent in our tests; they are smaller than CS-1, a 25-amino acid peptide, and therefore easier to synthesise and; cyclic peptides are more stable to enzymic degradation.

The compound number 1 (Table 2) is an especially preferred compound as are compound numbers 11 to 14. Especially preferred compounds have shown activity in in
15 vivo screens [in the mouse in vivo CHS (contact hypersensitivity) model] - See Example 2 below. CS1 at 10mg/kg/day and 1mg/kg/day gave 0% inhibition. No toxicity was observed for compounds tested of the present invention at the effective dose.

According to a further feature of the invention there is provided a pharmaceutical composition which comprises a cyclic dipeptide of the invention in
20 association with a pharmaceutically acceptable diluent or carrier.

The composition may be in a form suitable for oral use, for example a tablet, capsule, aqueous or oily solution, suspension or emulsion; for nasal use, for example a snuff, nasal spray or nasal drops; for vaginal or rectal use, for example a suppository; for administration by inhalation, for example as a finely divided powder or a liquid aerosol; for
25 sub-lingual or buccal use, for example a tablet or capsule; or for parenteral use (including intravenous, subcutaneous, intramuscular, intravascular or infusion), for example a sterile aqueous or oily solution or suspension. The composition may be in a form suitable for topical administration such as for example creams, ointments and gels. Skin patches are also contemplated. Formulation in general is described in Chapter 25.2 of Comprehensive
30 Medicinal Chemistry, Volume 5. Editor Hansch et al, Pergamon Press 1990.

In general the above compositions may be prepared in a conventional manner using conventional excipients. However, in the case of a composition for oral administration, it may be convenient for the composition to include a coating to protect the cyclic dipeptide active ingredient from the actions of enzymes in the stomach.

5 A preferred composition of the invention is one suitable for oral administration in unit dosage form for example a tablet or capsule which contains from 2.5 to 500 mg, and preferably 10 to 100 mg, of cyclic dipeptide in each unit dose, or one suitable for parenteral administration which contains from 0.5 to 100 mg of cyclic dipeptide per ml, and preferably 1 to 10 mg of cyclic dipeptide per ml of solution.

10 A parenteral composition is preferably a solution in isotonic saline or isotonic dextrose buffered if necessary to a pH of 5 to 9. Alternatively, the parenteral composition may be one designed for slow release over a period of at least 5 days, in which case the amount of polypeptide per unit dose is in general greater than that required when a conventional injectable formulation is used. A preferred slow release formulation is a
15 continuous release formulation, for example a formulation of the type described in European Patent Specification No. 58481. A preferred slow release parenteral formulation contains from 10 to 100 mg of cyclic dipeptide per unit dose.

The composition of the invention will normally be administered such that a daily oral dose will be from 0.1 mg/kg, to 50 mg/kg and a daily parenteral dose, will be
20 from 20 micrograms/kg to 10 mg/kg.

Accordingly, the invention provides a cyclic dipeptide as herein described for use as a medicament.

According to a further feature of the invention there is provided a method for inhibiting the interaction between VCAM-1 and/or fibronectin and the integrin receptor
25 VLA-4 in warm-blooded animals i.e. mammals such as man in need of such treatment which comprises administering to said mammal an effective amount of a cyclic dipeptide of formula 1 or a pharmaceutically acceptable salt thereof. In a preferred embodiment the mammal in need of treatment is suffering from multiple sclerosis, asthma or rheumatoid arthritis. The invention also provides the use of such a cyclic dipeptide of formula 1 or a
30 pharmaceutically-acceptable salt thereof in the production of a new medicament for use in

the treatment of a disease or medical condition mediated by the interaction between VCAM-1 and the integrin receptor VLA-4.

Synthetic Details

5 A cyclic dimeric peptide of the invention of formula 1 may be prepared by any process well known in the art of peptide chemistry to be applicable to the synthesis of analogous compounds. Thus, for example, a cyclic dipeptide of the invention may be obtained by procedures analogous to those disclosed in "Solid Phase Peptide Synthesis: A practical approach" by Atherton and Sheppard (published by IRL press at Oxford
10 University Press, 1989), "Solid Phase Peptide Synthesis" by Stewart and Young (published by the Pierce Chemical Company, Illinois, 1984), "Principles of Peptide Synthesis" (published by Springer-Verlag, Berlin, 1984) "Practice of Peptide Synthesis" (published by Springer-Verlag, Berlin, 1984), and a series of books "Amino Acids, Peptides and Proteins" (volumes 1-25; volume 25 published in 1994) (published by the Royal Society of
15 Chemistry, Cambridge, U.K.). Synthesis may be achieved by automated or manual means.

Preferred processes for the manufacture of a cyclic dipeptide of the invention include:-

- (a) The removal of one or more conventional peptide protecting groups from a protected cyclic dipeptide of Formula 10 (Figure 10) wherein Pr1 represents a protecting
20 group on the acid group in the side chain of AA3 to give a cyclic dipeptide of the invention of formula I and optionally, simultaneously or subsequently, also removing any additional conventional peptide protecting groups;
- (b) The formation of an amide bond by coupling two peptide units, one containing a carboxylic acid group, or a reactive derivative thereof, and the other containing an amino
25 group, such that a protected or unprotected cyclic dimeric peptide having the sequence indicated in formula I is produced, if necessary, the protecting groups are removed using process (a) above.

The invention will now be illustrated by the following non-limiting examples in which

30 Figure 1 illustrates Formula 1

Figure 2 illustrates peptides 1 & 2 in antiparallel juxtaposition.

- 11 -

Figure 3 illustrates peptides 1 & 2 in parallel juxtaposition.

Figure 4 illustrates Formulas 4-9

Figure 5 illustrates synthesis of compound 5 (Table 2)

Figure 6 illustrates synthesis of compound 8 (Table 2).

5 Figure 7 illustrates preferred linkers for antiparallel juxtapositions of peptides 1 & 2

Figure 8 illustrates analogues of Ile.

Figure 9 illustrates Tic (1) and nipecotic acid (2)

Figure 10 illustrates Formula 10

Figure 11 illustrates a detailed structure of compound 1 (Table 2)

10 Figure 12 illustrates preferred linkers for parallel juxtapositions of peptides 1 & 2

Figure 13 illustrates synthesis of compound 1 (Table 2)

Figure 14 illustrates synthesis of compound 1 (Table 2), second route

Figure 15 illustrates synthesis of compound 3 (Table 2)

Figure 16 illustrates synthesis of compound 4 (Table 2)

15 Figure 17 illustrates preferred linkers

In the Figures arrowed bonds indicate attachment points only (ie not a -CH₂- group). Illustrations of attachment points will be such that a nitrogen atom will link to a -C(O)- and vice versa unless otherwise stated or implicit.

20

Table 1 illustrates synthesis and purification of cyclic dipeptides.

Table 2 illustrates characterisation of cyclic dipeptides.

Abbreviations:

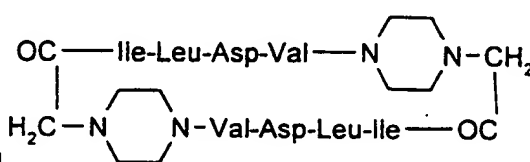
25

Boc	<u>tert</u> -butoxycarbonyl
Cha	cyclohexylalanine
Dab	2,4-diamino-butyric acid
Fmoc	9-fluorenylmethoxycarbonyl
30 Nle	norleucine
Orn	ornithine

Pbf	2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulphonyl
Tic	1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid
Z	benzyloxycarbonyl

5 All temperatures are in degrees centigrade unless otherwise stated.

Example 1 - Synthesis of Compounds 1-16 (Table 2)



1. Synthesis of Compound 1

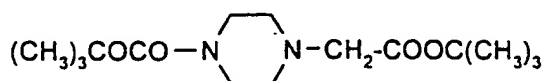
(Figure 13)

10

The cyclic dipeptide was prepared by two different routes. In each of these methods, the required linear peptide was prepared by solid phase peptide synthesis techniques using 2-chlorotritylchloride resin. The synthetic schemes are shown in figures 13 and 14 and the details are described below in this section and in section 2. After
 15 assembling the protected peptide on the resin, the linear peptide was cleaved from the resin and used in the subsequent steps without any purification. However, the final product was purified extensively by reverse phase high pressure liquid chromatography (HPLC) before characterisation.

20

1.1. Synthesis of



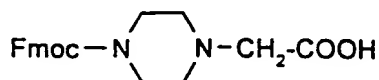
(Figure 13, Step 1)

t-Butyl bromoacetate (4.88 g, 25 mmols) in dichloromethane (50 ml) was added to a solution of t-butyl-1-piperazine carboxylate (4.65 g, 25 mmols) and triethylamine (3.5
 25 ml, 25 mmols) in dichloromethane (30 ml). The reaction mixture was stirred overnight, filtered to remove the solids separated overnight and the filtrate evaporated to dryness. The residue was partitioned between ethyl acetate and water, the organic layer was then washed

- 13 -

with water, dried over MgSO_4 and evaporated to dryness. The residue was crystallised from ether-isohexane to yield the product (5.66 g, 75%, m.p. 99-100 °C). [Elemental analysis: Found C 59.8%, H 9.6%, N 9.1%; $\text{C}_{15}\text{H}_{28}\text{N}_2\text{O}_4$ requires C 60.0%, H 9.4%, N 9.33%]. [Thin layer chromatography on silica gel plates showed a single spot; R_f 0.38 in ethyl acetate-isohexane (1:1) and 0.68 in methanol-chloroform (1:9)].

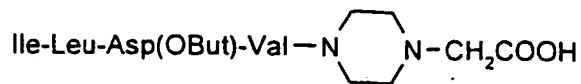
1.2. Synthesis of



(Figure 13, Step 2)

The compound described in section 1.1 (5 g, 16.6 mmols) was treated with a mixture of trifluoroacetic acid-water (95:5; 50 ml) for 1 hour. The acid was removed by evaporation in vacuo and the residual oil was triturated with ether to give a solid which was collected, washed with ether and dried over $\text{P}_2\text{O}_5/\text{KOH}$ under vacuum (6.25 g, m.p. 177-182 °C). The solid was then dissolved in a mixture of water and acetone (1:1, 150 ml) containing potassium carbonate (6.92g, 3 equivalents). 9-Fluorenylmethyl-N-hydroxysuccinimide (5.66 g, 16.7 mmols) in acetone (30 ml) was added over a period of 20 minutes with stirring. The pH of the solution was maintained at about 9 by the addition of M K_2CO_3 solution. After stirring overnight at room temperature, the acetone was removed by evaporation under vacuum and the aqueous solution was acidified with KHSO_4 solution. The product was extracted into ethyl acetate and the solution was washed with water (6 times) and with saturated NaCl solution. The organic layer was dried over MgSO_4 and evaporated to give an oil which solidified on trituration with isohexane and ether (yield 3.72 g, 60%). A sample was recrystallised from ethanol-ether, m.p. 179-182 °C, MH^+ 367.

1.3. Synthesis of



(Figure 13, Steps 3 and 4)

- 14 -

The Fmoc-piperazine derivative (366 mg, 1 mmole) in dichloromethane (15 ml) and diisopropylethylamine (525 μ l, 3 equivalents) was added to 2-Chlorotritylchloride resin (Nova Biochem., 1g) and the reaction mixture was shaken gently for 75 minutes. A 10% solution of diisopropylethylamine in methanol (10 ml) was added and the shaking was continued for 10 minutes.. The resin was filtered off, washed successively with methylene chloride, dimethylformamide, methylene chloride, ether and dried at 50 °C in a vacuum oven (weight 1.13 g).

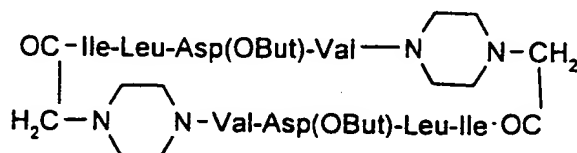
The above resin was placed in a reaction vessel fitted with a sintered glass disc. The following series of reactions were then carried out manually to obtain the desired peptide resin.

- (a) Removal of the Fmoc group with two treatments (1 x 5 minutes and 1 x 15 minutes) of 20% piperidine in dimethylformamide.
- (b) Dimethylformamide washes (5).
- (c) Acylation with Fmoc-Val (678 mg, 2 mmole) activated with O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (760 mg, 2 mmole) and diisopropylethylamine (700 μ l, 4 mmole) in dimethylformamide (4 ml) for 1 hour.
- (d) Dimethylformamide washes (5)

The above deprotection and coupling cycles were repeated using Fmoc-Asp(OBu^t) (822 mg, 2 mmoles), Fmoc-Leu (700 mg, 2 mmoles) and Fmoc-Ile (700 mg, 2 mmoles) to give the protected tetrapeptide derivative attached to the chlorotrityl resin. The N-terminal Fmoc group was cleaved with 20% piperidine in dimethylformamide (1 x 5 minutes and 1 x 15 minutes) and the peptide resin was washed successively with dimethylformamide, methylene chloride and ether and dried in a vacuum oven at 50 °C.

- 15 -

The peptide resin was suspended in a mixture of acetic acid-trifluoroethanol-methylene chloride (2:2:6) (25 ml) for 2 hours. The resin was removed by filtration, washed with the above solvent mixture. The combined filtrates were evaporated and the residue triturated with ether to give the linear tetrapeptide derivative as an acetate salt. The acetate salt was then converted to a hydrochloride salt by dissolving it in a mixture of water-acetonitrile (2:1, 60 ml), cooling to 0 °C, adding 1.05 equivalents of 1N HCl and freeze drying the contents.

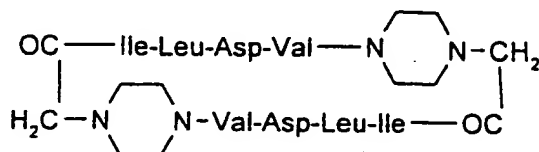


1.4. Synthesis of

(Figure 13, Step 5)

10

The linear peptide hydrochloride (388 mg, 0.57 mmole) was dissolved in dimethylformamide (600 ml) and O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (218 mg, 0.57 mmoles), was added followed by diisopropylethylamine (300 µl, 1.72 mmoles). The reaction mixture was stirred for 24 hours at room temperature and then evaporated to dryness under vacuum.



1.5. Synthesis of

(Figure 13, Step 6)

The crude cyclic dipeptide was treated for 1 hour with a mixture of trifluoroacetic acid-water (95:5, 25 ml) and triisopropylsilane (200 µl) to remove the aspartic acid side chain protecting groups. Evaporation to a small volume, followed by trituration with ether yielded the crude cyclic peptide which was purified by preparative reverse phase HPLC on a Vydac 218TP1022 column using a gradient of acetonitrile-water containing 0.1% trifluoroacetic acid (20-50%) over a period of 65 minutes at a flow rate of 10.0 ml/minute. The product-containing fractions were combined and freeze dried to give the purified

- 16 -

cyclic peptide (46 mg). The peptide was characterised by amino acid analysis and mass spectroscopy.

5 **2. Synthesis of Compound 1 (Second route, Figure 14).**

The second route shown in figure 14 involved the synthesis of a linear octapeptide derivative (structure shown below) in place of the tetrapeptide derivative used in the first route. The first three steps in both routes 1 and 2 are the same.

10

2.1. Synthesis of Compound 1 (Steps 4 and 5, Figure 14).

The Fmoc protected tetrapeptide resin obtained in step 3 (figure 14) was divided in two parts. One half of the resin was treated in the standard manner with piperidine to
15 cleave the N-terminal Fmoc group to give the partially protected tetrapeptide derivative still attached to the resin (step 4). The other half was treated in a standard fashion with a mixture of acetic acid-trifluoroethanol-methylene chloride (2:2:6) to cleave the Fmoc Protected tetrapeptide derivative from the resin (step 5).

20 The Fmoc protected fragment (800 mg, 0.93 mmole) was then coupled to the tetrapeptide fragment still attached to the resin by the standard method (HBTU, 352 mg, 0.93 mmole, diisopropylethylamine 325 μ l, 1.85 mmole) used to couple various amino acid derivatives during the chain elongation process.

25 **2.2. Synthesis of Compound 1 (Steps 6, 7 and 8, Figure 14).**

The linear octapeptide derivative assembled on the resin was deblocked at the N-terminal end (Fmoc cleavage), cleaved from the resin, cyclised and deprotected [Asp(OBut) cleavage] by the standard methods used in the first route. The dimeric peptide
30 was then purified by using the solvent system described in table 1 (yield 374 mg).

3. Synthesis of Compound 2

The peptide was prepared by the first route described above for the corresponding
5 Ile containing compound 1. HPLC analysis indicated the product to be a mixture of the
required dimer (38%) and a cyclic monomer (62%). Reverse phase HPLC purification
using the solvent system described in table 1 gave the desired dimeric peptide which was
characterised by amino acid analysis and mass spectroscopy (table 2).

10

4. Synthesis of Compound 3 (Figure 15)

The linear peptide required for the synthesis of this cyclic dipeptide (table 1) was
assembled on the 2-chlorotritylchloride resin. 3-Bromopropionic acid was reacted with the
15 resin in a manner similar to that described above for the Fmoc-piperazine derivative in
example 1.3 (compound 1). A five-fold excess of piperazine was then added to the 3-
bromopropionyl-O-(2-chlorotrityl)-resin to give piperazine-N-propionyl derivative linked
to the resin (structure shown below in step 1, Figure 15)

20 Fmoc-Val, Fmoc-Asp(OBut), Fmoc-Leu and Fmoc-Ile were then coupled to the
resin by the procedures described in section 1.3 to give the Fmoc-tetrapeptide piperazinyl
derivative. Piperidine treatment in a standard manner to cleave the N-terminal Fmoc group
gave the partially protected tetrapeptide derivative shown in step 2 (figure 15). Further
coupling and deprotection reactions using 3-bromopropionic acid, piperazine, Fmoc-Val,
25 Fmoc-Asp(OBut), Fmoc-Leu and Fmoc-Ile by the standard procedures gave the
octapeptide derivative containing two piperazine groups (step 4, figure 15). The peptide
was then cleaved from the resin, cyclised, deprotected and purified by the procedures
described above for compound 1.

30

5. Synthesis of Compounds 4 and 5

Both these compounds were synthesised by the second route described above for the synthesis of compound 1 (figure 14). As shown in figures 16 and 5, one of the fragments with a free amino group at the N-terminus was left attached to the resin and a second fragment containing an Fmoc group at the N-terminus was cleaved from the resin. The second fragment containing a C-terminal carboxyl group was activated by the HBTU method and coupled to the fragment still attached to the resin.

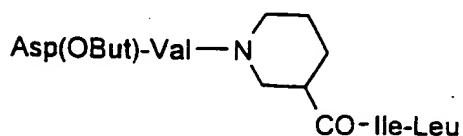
The linear peptide derivatives assembled on the resin were deblocked at the N-terminal end (Fmoc cleavage), cleaved from the resin, cyclised and deprotected [Asp(OBut) cleavage] by the standard methods used in the synthesis of compound 1. The dimeric peptides were then purified by using the solvent systems described in table 1.

6. Synthesis of Compound 6

Starting from β -alanine, the linear decapeptide, D-Leu-Leu-Asp(OBut)-Val- β -Ala-D-Leu-Leu-Asp(OBut)-Val- β -Ala, was assembled on the chlorotritylresin. The rest of the synthesis (cleavage, cyclisation and deprotection) was similar to that of compound 1.

7. Synthesis of Compound 7

7.1. The peptide was prepared by the dimerisation of the linear peptide (structure shown below) which was assembled on the 2-chlorotritylchloride resin by the method described below.



- 19 -

A solution of Fmoc-Leu (700 mg, 2 mmole) and diisopropylethylamine (1.05 ml, 6 mmole) in dry dichloromethane (20 ml) was added to a suspension of 2-chlorotriylchloride resin (2 g) in dichloromethane (10 ml), and the reaction mixture was shaken gently for 75 minutes. A 10% solution of diisopropylethylamine in methanol (10 ml) was added and shaking was continued for a further 10 minute period. The resin was filtered off, washed successively with dichloromethane, dimethylformamide, dichloromethane and ether and dried at 50 °C in a vacuum oven (weight 2.4 g).

The above resin was placed in a reaction vessel fitted with a sintered glass disc.

10 The following series of reactions were then carried out manually to obtain the desired peptide resin.

(a) Removal of the Fmoc protecting group with two treatments (1 X 5 min., 1 X 15 min.) of 20% piperidine in dimethylformamide followed by five washes with dimethylformamide.

15

(b) Acylation of the Leu resin with Fmoc-Ile (1.42 g, 4 mmole) activated with O-(benzotriazol-1-yl),1,1,3,3,-tetramethyluronium hexafluorophosphate (HBTU) (1.52 g, 4 mmole) and diisopropylethylamine (1.4 ml, 8 mmole) in dimethylformamide (8 ml) for 30 minutes. After the reaction, the resin was washed five times (as above) with dimethylformamide to remove the excess reagents and dried. Only half of the Fmoc-Ile-Leu resin thus obtained was used in the next step.

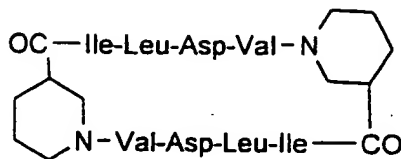
20

(c) The above deprotection and coupling cycles (a and b) were repeated using Fmoc-nipicotic acid (702 mg, 2 mmole), Fmoc-Val (678 mg ,2 mmole) and Fmoc-Asp(OBut) (822 mg, 2 mmole) to give the required partially protected tetrapeptide attached to the resin. The peptide was cleaved from the resin by two treatments with acetic acid-trifluoroethanol-dichloromethane (2:2:6; 15 ml) for 1 hour. The resin was removed by filtration and washed with the above deblocking solvent mixture. The combined filtrates were evaporated and the residue triturated with ether to give the linear partially protected tetrapeptide as an acetate salt. The peptide was converted to a hydrochloride salt by

25

30

dissolving in water-acetonitrile (2:1; 60 ml), cooling to 0°C, adding 1.05 equivalents of 1N HCl and freeze drying.



7.2. Synthesis of Compound 7

5

The linear peptide hydrochloride (350 mg, 0.51 mmole) was dissolved in dimethylformamide (500 ml) and HBTU (194 mg, 0.51 mmole) was added followed by diisopropylethylamine (268 μ l, 1.53 mmole). The reaction was monitored by reverse phase HPLC. After 3 hours, when no starting material could be detected, the reaction mixture
 10 was evaporated to dryness and the residue treated with a mixture of trifluoroacetic acid-water (95:5; 25 ml) and triisopropylsilane (500 μ l) for one hour. After evaporation to low volume ether was added and the crude product collected by filtration. HPLC analysis indicated the product to be a mixture of the required dimer (72%) and a cyclic monomer (28%). Reverse phase HPLC purification using the solvent system described in table 1
 15 gave the desired dimeric peptide which was characterised by amino acid analysis and mass spectroscopy (table 2).

8. Synthesis of Compound 8, c(D-Arg-Melle-Leu-Asp-Val-D-Arg-Melle-Leu-Asp-Val), (Figure 6)

20

The cyclic peptide was prepared by the solid phase procedure using 2-chlorotritylchloride resin. The synthetic details are described below. After assembling the partially protected linear peptide on the resin, the peptide was cleaved from the resin and used in the subsequent steps without any purification. However, the final product was
 25 purified extensively by reverse phase high pressure liquid chromatography (HPLC) before characterisation.

8.1. Preparation of Fmoc-Val-chlorotrityl resin (step 1, Figure 6)

- 21 -

2-Chlorotritylchloride resin (Alexix Corporation: 1.35 mmole Cl/g; 5g) was swollen in dichloromethane (30 ml) (dried over molecular sieve) for 5 minutes. A solution of Fmoc-Val (1.7 g, 5 mmole) and diisopropylethylamine (2.63 ml, 15 mmole) in
5 dichloromethane (25 ml) was added and the suspension was shaken mechanically for 45 minutes. Methanol (9 ml) and diisopropylethylamine (1 ml) were added and the shaking was continued for a further five minute period. The resin was collected by filtration and washed successively with dichloromethane, dimethylformamide, dichloromethane, isopropanol and ether, and finally dried at 50 °C in a vacuum oven (weight 5.25 g)

10

8.2. Preparation of D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val-D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val-chlorotrityl resin (steps 2 and 3, Figure 6)

The above Fmoc-Val resin (2 g) was placed in a reaction vessel fitted with a
15 sintered glass disc. The following series of reactions were then carried out manually to obtain the desired peptide resin.

(a) Removal of the Fmoc group with two treatments (1 x 5 minutes and 1 x 15 minutes) of 20% piperidine in dimethylformamide followed by five washes with dimethylformamide to
20 remove excess reagents and cleavage products.

(b) Acylation with Fmoc-Asp(OBu^t) (2.06 g, 5 mmole), activated with O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (1.90 g, 5 mmole) and diisopropylethylamine (1.75 ml, 10 mmole) in dimethylformamide (8 ml) for 1 hour. The
25 resin was again washed five times with dimethylformamide to remove excess reagents.

The above deprotection and coupling cycles were repeated using Fmoc-Leu (1.78 g, 5 mmole), Fmoc-Melle (1.84 g, 5 mmole), Fmoc-D-Arg(Pbf) (3.76 g, 5 mmole) to give Fmoc-D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val-chlorotritylresin. Further elongation of this
30 pentapeptide resin to give the protected decapeptide resin was carried out using one half of the resin. The deblocking and coupling reactions on this pentapeptide resin were continued

using Fmoc-Val (850 mg, 2.5 mmole), Fmoc-Asp(OBut) (1.03 g, 2.5 mmole), Fmoc-Leu (855 mg, 2.5 mmole), Fmoc-Melle (920 mg, 2.5 mmole), Fmoc-D-Arg(Pbf) (1.87 g, 2.5 mmole). As in the case of compound 1, coupling of the Fmoc-D-Arg derivative was achieved by using O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium

- 5 hexafluorophosphate (HATU) and diisopropylethylamine. The N-terminal Fmoc group was cleaved (step 3) with 20% piperidine in dimethylformamide (1 x 5 minutes and 1 x 15 minutes) and the peptide resin, D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val-D-Arg(Pbf)-Melle-Leu-Asp(OBut)-Val-chlorotrityl resin, was washed successively with dimethylformamide, dichloromethane and ether and dried in a vacuum oven at 50 °C.

10

8.3. Preparation of D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val-D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val, HCl. (step 4, Figure 6)

- The peptide resin, D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val-D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val-chlorotrityl resin, was suspended in a mixture of acetic acid-trifluoroethanol-dichloromethane (2:2:6) (25 ml) for 1 hour. The resin was removed by filtration and retreated with the same mixture for a further one hour. The combined filtrates were evaporated and the residue triturated with ether to give D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val-D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val as an acetate salt (1.34 g).
- 15 The acetate salt was then converted to a hydrochloride salt by dissolving it in a mixture of water-acetonitrile (2:1, 60 ml), cooling to 0 °C, adding 1.05 equivalents of 1N HCl and freeze drying the contents.
- 20

8.4. Preparation of c(D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val-D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val) (step 5, Figure 6)

- The above linear peptide hydrochloride, D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val-D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val (HCl), (1.34 g, 0.7 mmole) was dissolved in dimethylformamide (700 ml) and O-(7-aza-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium
- 30 hexafluorophosphate (HATU) (264 mg, 0.7 mmole), and diisopropylethylamine (370 µl, 2.1 mmole) were added to the solution. The cyclisation reaction was monitored by

- 23 -

analytical HPLC. On completion of the reaction (2 hours at room temperature), the reaction mixture was evaporated to dryness in vacuum, ether was added and the solid collected by filtration. The product [retention time 22.06 minutes on a Vydac 218TP54 column using a gradient of acetonitrile-water containing 0.1% trifluoroacetic acid (60-95%) over a period of 30 minutes at a flow rate of 1.0 ml/minute] was used in the next step without any purification.

8.5. Preparation of c(D-Arg-Melle-Leu-Asp-Val-D-Arg-Melle-Leu-Asp-Val) [compound 8] (step 6, Figure 6)

10

The above protected cyclic peptide, c(D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val-D-Arg(Pbf)-Melle-Leu-Asp(OBu^t)-Val) was treated for 150 minutes with a mixture of trifluoroacetic acid-water (95:5, 30 ml) and triisopropylsilane (1 ml) to remove the arginine and aspartic acid side chain protecting groups. Evaporation to a small volume, followed by trituration with ether yielded the crude cyclic peptide (1.06 g). The crude product was purified by preparative reverse phase HPLC on a Deltapak C₁₈ column (30x30 mm) using a gradient of acetonitrile-water containing 0.1% trifluoroacetic acid (20-36%) over a period of 80 minutes at a flow rate of 30.0 ml/minute. The product-containing fractions were combined and freeze dried to give the purified cyclic peptide (280 mg). The peptide [single peak on HPLC, retention time 29.60 minutes on a Vydac 218TP54 column using a gradient of acetonitrile-water containing 0.1% trifluoroacetic acid (20-45%) over a period of 30 minutes at a flow rate of 1.0 ml/minute] was characterised by amino acid analysis and mass spectroscopy (table 2).

25 9. Syntheses of Compounds 9 and 10

Both these compounds were synthesised by the same procedure as described above for compound 8.

30

10. Syntheses of Compounds 11 to 14

The peptides were synthesised by the second route used for compound 1 (section 2, figure 14). The linear octapeptide derivatives for compounds 11, 12 and 14 and a
5 decapeptide derivative for compound 13 (sequences shown in table 1) were first assembled on the resin followed by cleavage, cyclisation and deprotection by the methods described above to give the required products.

11. Synthesis of Compound 15

10

Compound 15 was synthesised by the same procedure as described above for compound 8 except that the unusual linking group $\text{-NH(CH}_2\text{)}_2\text{-S-CH}_2\text{-CO-}$ was incorporated by using $\text{Fmoc-NH(CH}_2\text{)}_2\text{-S-CH}_2\text{-COOH}$. Synthesis of this derivative is described below.

15

$\text{Fmoc-NH(CH}_2\text{)}_2\text{-S-CH}_2\text{-COOH}$ (used above) was obtained from 2-aminoethanethiol and 2-bromoacetic acid. 2-Aminoethanethiol hydrochloride (5.68 g, 50 mmole) was dissolved in water (200 ml) and sodium hydrogen carbonate (25.2 g, 300 mmole) was added to it. 2-Bromoacetic acid (6.95 g, 50 mmole) dissolved in acetonitrile (100 ml) was added in portions over 30 minutes to the stirred solution prepared above.
20 After 1 hour at room temperature, a solution of 9-fluorenylmethyl-N-hydroxysuccinimide (Fmoc-OSu) (16.85 g, 50 mmole) in acetonitrile (150 ml) was added and the stirring was continued for 16 hours. The slightly turbid solution was evaporated to remove most of the acetonitrile and the remaining aqueous solution was extracted with ethyl acetate (3 X 50 ml) and acidified (pH 2) by the addition of hydrochloric acid. The white solid was
25 collected, washed with water and dried in vacuo at 45 °C. Yield 17 g (95%), $(\text{M}+\text{H})^+$ 358.0.

12. Synthesis of Compound 16

30

Compound 16 was synthesised by the same procedure as described above for compound 8.

Example 2 - In Vitro and In Vivo Assays

The following abbreviations and sources of materials are used in the following
5 examples.

MOLT-4 cells - lymphocytic T cell line (ATCC derived)

Fibronectin - Reagent grade human fibronectin. Purified from human plasma by gelatin-sepharose affinity chromatography. Source: Bio Products Elstree UK. Product No. 9136. A review article on fibronectins is Fibronectins - Adhesive Glycoproteins of Cell

10 Surface and Blood, K.M. Yamada and K. Olden, Nature, 275 (1978) 179-184.

rsVCAM-1 - (Reference source: Biochem Biophys Res Comm 1991 178 N3; 1498-1504).

VCAM-1 is a cell surface glycoprotein produced by the vascular endothelium, as well as on macrophage-like and dendritic cell types, in response to certain inflammatory stimuli.

VCAM-1 interacts with the integrin VLA-4 present on mononuclear leukocytes.

15 The cDNA for VCAM-1 was isolated by screening a cDNA library from IL-1 β -activated human endothelial cells. Large quantities of the protein were expressed in insect cells using a baculovirus expression system. VCAM-1 expressing cells were shown to bind specifically to a variety of VLA-4 expressing cell lines (Jurkat, THP-1, U937).

Another reference on VCAM-1 is Expression and Functional Characterisation of

20 Recombinant Human Vascular Cell Adhesion Molecule-1 (VCAM-1) Synthesised by Baculovirus-Infected Insect Cells. J.K. Stoltenborg, R.A. Straney, R.J. Tritch, W.M. Mackin and H.J. George, Protein Expression and Purification, 4 (1993) 585-593.

RPMI 1640 - Cell media. Source Gibco BRL (Life technologies; Cat No 31870-025).

FCS - Foetal calf serum. Source Advanced protein products (West Midlands UK) Cat No
25 AS-302-50.

BCECF-AM - 2',7'-bis (2 carboxyethyl)-5-(ϵ 6)-carboxyfluorescein acetoxymethyl ester). source: Molecular Probes Inc USA; Cat No B-1150.

CHO DG44 - Chinese hamster ovary cell line (ATCC derived; Reference: Som Cell Mol Gen 1986; 12; 555-666)

30 DMEM - Dulbecco's modified eagle medium. Source Gibco BRL (Life technologies; Cat No 41966-029).

Antibiotic - Penicillin-streptomycin. Source Gibco BRL (Life Technologies; Cat No 15070-022).

Fluorskan - is a fluorimeter.

HUVEC - Human umbilical cord endothelial cells. Primary cultures prepared from tissue samples. (Reference: J Clin Invest. 1973 52; 2745-2747.

Recombinant human TNF α - Tumor necrosis factor

Alzet osmotic minipump - Subcutaneous implanted micro osmotic pump, Alza Corporation Palo Alto, California.

10 2.1 MOLT-4 cell/ Fibronectin-VCAM-1 Adhesion Assay.

The MOLT-4 cell /Fibronectin-VCAM-1 adhesion assay is used to investigate the interaction of the integrin VLA4 (Very Late Antigen, $\alpha 4/\beta 1$) expressed on the MOLT-4 cell membrane with fibronectin or recombinant soluble VCAM-1 (rsVCAM-1).

Fibronectin or rsVCAM-1 are coated overnight at 4°C onto polystyrene 96-well microtitre plates at concentrations of 20 μ g/ml and 1 μ g/ml respectively. Following this, a concentrated BSA solution (10 mg/ml) is added to block non-specific binding sites. After aspiration of these solutions, equal volumes of compound and MOLT-4 cell suspension (1 X 10⁶ cells/ml) are added. Adhesion takes place during a 2 hour incubation at 37°C, non or loosely adherent cells are removed by gentle agitation followed by vacuum aspiration. Quantitation of the remaining adherent cells is by means of a colorimetric assay of acid phosphatase activity, which is read on a spectrophotometer. Compounds which inhibit adhesion result in a lower absorbance reading. Standard, control and test conditions are assayed in triplicate, percentage inhibition being calculated with respect to total (no inhibitor) and non-specific (no fibronectin) standards on each plate.

25

2.2 Cell-Cell Assays

2.2.1. VCAM-1 CHO cells

MOLT-4 cells (RPMI 1640 supplemented with 5% FCS and 2mM L-Glutamine) are labelled with the fluorescent dye BCECF-AM (30 μ g/ml per 3 X 10⁶ cells). CHO DG44 transfected with full length VCAM-1 cDNA were selected for VCAM-1 expression by FACS analysis and grown to confluence in 96 well tissue culture plates. Prior to use in

- 27 -

the adhesion assay CHO DG44 cells are washed three times (DMEM supplemented with 5% FCS, 2mM L-Glutamine and 2% antibiotic). MOLT-4 (10^5 cell/well) cells are overlaid on the VCAM-1 expressing CHO cells and incubated for 30 minutes at 37°C, 5% CO₂. The non-adherent cells are removed by washing the plate three times (RPMI 1640 supplemented with 5% FCS and 2mM L-Glutamine) following which the plates are blotted dry on tissue paper. 100µl of 2% Triton X-100 is added to each well and the plates read using a Fluoroskan (excitation = 485nm, emission = 538nm). Compounds are dissolved in appropriate solvents and added to the MOLT-4 cells prior to addition to HUVEC cultures, inhibition of adhesion is calculated comparing level of adhesion (fluorescence) of control vehicle treated cells with compound treated cells.

2.2.2 Human Umbilical Vein Endothelial Cells.

MOLT-4 cells (RPMI 1640 supplemented with 5% FCS and 2mM L-Glutamine) are labelled with the fluorescent dye BCECF-AM (30µg/ml per 3×10^6 cells). Primary HUVEC are grown to confluence in 96 well tissue culture plates and incubated for 18 hours with 2 U/ml recombinant human TNFα. Prior to use in the adhesion assay the primary HUVEC monolayers are washed (M199 supplemented with 5% FCS, 2mM L-Glutamine and 2% antibiotic). MOLT-4 (10^5 cell/well) cells are overlaid on the primary HUVEC and incubated for 30 minutes at 37°C, 5% CO₂. The non-adherent cells are removed by washing the plate three times (RPMI 1640 supplemented with 5% FCS and 2mM L-Glutamine) and dried by blotting on tissue paper. 100µl of 2% Triton X-100 is added to each well and the plates read using a Fluoroskan (excitation = 485nm, emission = 538nm). Compounds are dissolved in appropriate solvents and added to the MOLT-4 cells prior to addition to HUVEC cultures, inhibition of adhesion is calculated comparing level of adhesion (fluorescence) of control vehicle treated cells with compound treated cells.

2.3 In Vivo Contact hypersensitivity Response.

Balb/C male mice (20-25g) are sensitised with oxazolone (50µl of 0.24% in acetone/olive oil) by topical application to the shaved skin area of the back. Seven days later the mice are challenged by topical application of oxazolone (25µl of 0.25% in acetone/olive oil) to the surface of the ear. Swelling of the ear develops over a 24 hour

period following which ear thickness is measured and compared to the pre-challenge thickness, the percentage increase in ear thickness is calculated. Compounds are delivered via Alzet osmotic minipump daily dosing (once/day) which are implanted 24 hours prior to the oxazolone challenge, inhibition of the inflammatory response is calculated comparing
5 vehicle treated animals and compound treated groups (n=6 animals per group).

2.4 In Vivo Ovalbumin Delayed type Hypersensitivity Model.

Balb/C female mice (20-25g) are immunised on the flank with an emulsion of ovalbumin (Sigma: 0.1 ml subcutaneous injection of 2 mg/ml solution mixed (1:1) with
10 complete Freund's adjuvant; Difco). Seven days later the mice are challenged by subplantar injection of ovalbumin (30 µl of 1% heat aggregated ovalbumin in saline) into the left hind foot pad. Swelling of the foot develops over a 24 hour period following which foot pad thickness is measured and compared to the pre-challenge thickness, the percentage increase in foot pad thickness is calculated. Compounds are delivered via Alzet osmotic
15 minipump daily dosing (once/day) which are implanted 24 hours prior to the ovalbumin challenge and the inhibition of the inflammatory response is calculated comparing vehicle treated animals and compound treated groups (n=5 animals per group).

2.5 In Vivo Antigen Induced Arthritis Model.

20 Mice are immunised and boosted 7 days later with a combination of 100 µg methylated BSA in complete Freund's adjuvant (s.c.) followed by an intraperitoneal injection of Bordetella pertussis organisms. Two weeks after boost animals are challenged with 100 µg methylated-bovine serum albumin (BSA) intra-articularly and the degree of inflammation/arthritis determined by measuring knee joint swelling, histology and changes
25 in acute phase proteins. Compounds are dosed for 7 to 14 days commencing the day prior to challenge and the degree of inflammation/arthritis compared with the control animals and contralateral knee.

2.6 Experimental Autoimmune Encephalomyelitis Model.

30 Disease induced by s.c. injection of a mixture of spinal cord homogenate, myelin basic protein (MBP) or encephalogenic peptides with complete Freund's adjuvant (CFA),

- 29 -

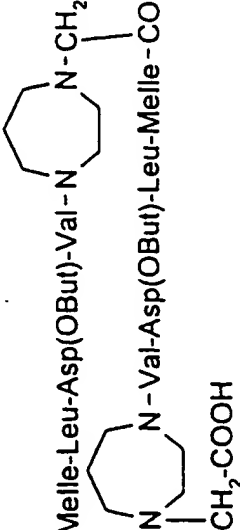
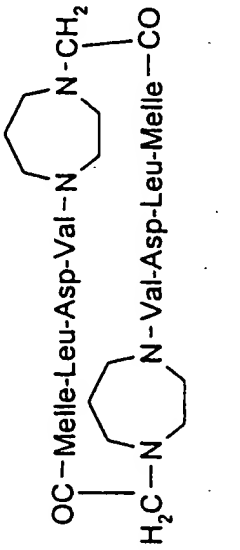
coupled with an i.p. injection of pertussis toxin. For acute disease, pertussis injection is repeated 2 days after immunisation. For chronic disease, pertussis is omitted and mice receive two injections of antigen in CFA, with an interval of 7 days. Disease is assessed by clinical scoring supported by histology. Compounds are dosed for 7 to 14 days
5 commencing the day prior to challenge and the symptoms compared with the control animals.

Table 1. Synthesis and purification of cyclic dimeric peptides.

Comp. No.	Precursor	No.	End Product Cyclic Peptide	High Pressure Liquid Chromatography (HPLC) (Gradient system and time)
17	Ile-Leu-Asp(OBut)-Val-N(CH ₂) ₆ -N-CH ₂ COOH	1	OC-Ile-Leu-Asp-Val-N(CH ₂) ₆ -N-CH ₂ -CO	20-50% water-acetonitrile (65 min.)
18	OR CO-Ile-Leu-Asp(OBut)-Val-N(CH ₂) ₆ -N-CH ₂ COOH CH ₂ -N(CH ₂) ₆ -N-Val-Asp(OBut)-Leu-Ile-CH ₂ COOH	2	OC-D-Leu-Leu-Asp-Val-N(CH ₂) ₆ -N-CH ₂ -CO	10-40% water-acetonitrile (60 min.)
19	D-Leu-Leu-Asp(OBut)-Val-N(CH ₂) ₆ -N-CH ₂ COOH	3	H ₂ C-CO-Ile-Leu-Asp-Val-N(CH ₂) ₆ -N-CH ₂ -CH ₂ H ₂ C-N(CH ₂) ₆ -N-Val-Asp-Leu-Ile-CO-CH ₂	10-50% water-acetonitrile (60 min.)
20	Ile-Leu-Asp(OBut)-Val-N(CH ₂) ₆ -N-CH ₂ CH ₂ H ₂ C-N(CH ₂) ₆ -N-Val-Asp(OBut)-Leu-Ile-CH ₂ COOH			

21	$\text{b-Ala-Ile-Leu-Asp(OBut)-Val-N} \begin{array}{c} \diagup \text{N-CH}_2 \\ \diagdown \text{CH}_2\text{-COOH} \end{array}$	4	$\text{CH}_2\text{CO-bAla-Ile-Leu-Asp-Val-N} \begin{array}{c} \diagup \text{N-CH}_2 \\ \diagdown \text{N-Val-Asp-Leu-Ile-bAla-CO-CH}_2 \end{array}$	20-45% water-acetonitrile (65 min.)
22	$\text{b-Ala-Ile-Leu-Asp(OBut)-Val-N} \begin{array}{c} \diagup \text{N-CH}_2 \\ \diagdown \text{CH}_2\text{-COOH} \end{array}$	5	$\text{CO-bAla-Ile-Leu-Asp-Val-N} \begin{array}{c} \diagup \text{N-CH}_2 \\ \diagdown \text{CH}_2\text{-N-Val-Asp-Leu-Ile-CO} \end{array}$	20-45% water-acetonitrile (65 min.)
23	D-Leu-Leu-Asp-Val-β-Ala-D-Leu-Leu-Asp-Val-β-Ala	6	$\text{OC-} \begin{array}{c} \diagup \text{CH}_2\text{-CH}_2\text{-NH-Val-Asp-Leu-D-Leu-CO} \\ \diagdown \text{CH}_2\text{-CH}_2\text{-NH-Val-Asp-Leu-D-Leu-CO} \end{array}$	10-50% water-acetonitrile (60 min.)
24	$\text{Asp(OBut)-Val-N} \begin{array}{c} \diagup \text{N-CH}_2 \\ \diagdown \text{CO-Ile-Leu} \end{array}$	7	$\text{OC-Ile-Leu-Asp-Val-N} \begin{array}{c} \diagup \text{N-CH}_2 \\ \diagdown \text{N-Val-Asp-Leu-Ile-CO} \end{array}$	15-55% water-acetonitrile (65 min.)
25	D-Arg(Pbf)-Melle-Leu-Asp(OBut)-Val-D-Arg(Pbf)-Melle-Leu-Asp-Val	8	c(Melle-Leu-Asp-Val-D-Arg-Melle-Leu-Asp-Val-D-Arg)	Deltapak column 20-36% (80 min.)

26	D-Arg(Pbf)-D-Arg(Pbf)-Melle-Leu-Asp(OBt)-Val-D-Arg(Pbf)-D-Arg(Pbf)-Melle-Leu-Asp-Val	9	c(Melle-Leu-Asp-Val-D-Arg-D-Arg-Melle-Leu-Asp-Val-D-Arg-D-Arg)	Deltapak column 10-40% (80 min.)
27	D-Ala-D-Ala-Melle-Leu-Asp(OBt)-Val-D-Arg(Pbf)-D-Arg(Pbf)-Melle-Leu-Asp-Val	10	c(Melle-Leu-Asp-Val-D-Arg-D-Arg-Melle-Leu-Asp-Val-D-Ala-D-Ala)	Deltapak column 20-36% (80 min.)
28	Melle-Leu-Asp(OBt)-Val-N(CH ₂) ₄ -N(CH ₂) ₄ -COOH	11	OC-Melle-Leu-Asp-Val-N(CH ₂) ₄ -N(CH ₂) ₄ -COOH	Deltapak column 20-40% (80 min.)
29	MePhe-Leu-Asp(OBt)-Val-N(CH ₂) ₄ -N(CH ₂) ₄ -COOH	12	OC-MePhe-Leu-Asp-Val-N(CH ₂) ₄ -N(CH ₂) ₄ -CO	Deltapak column 20-45% (80 min.)
30	D-Arg(Pbf)-MePhe-Leu-Asp(OBt)-Val-N(CH ₂) ₄ -N(CH ₂) ₄ -COOH	13	OC-D-Arg-MePhe-Leu-Asp-Val-N(CH ₂) ₄ -N(CH ₂) ₄ -CO	Deltapak column 20-30% (60 min.)

31	$\begin{array}{c} \text{Melle-Leu-Asp(OBut)-Val-N} \\ \\ \text{N} \\ \\ \text{CH}_2\text{-COOH} \end{array}$ 	14	$\begin{array}{c} \text{OC-Melle-Leu-Asp-Val-N} \\ \\ \text{H}_2\text{C-N} \\ \\ \text{N-Val-Asp-Leu-Melle-CO} \end{array}$ 	Deltapak column 20-45% (80 min.)
32	$\begin{array}{c} \text{NH}_2(\text{CH}_2)_2\text{-S-CH}_2\text{-CO-Melle-Leu-Asp(OBut)-} \\ \text{Val-NH-(CH}_2)_2\text{-S-CH}_2\text{-CO-Melle-Leu-} \\ \text{Asp(OBut)-Val} \end{array}$	15	$\begin{array}{c} \text{c(NH-(CH}_2)_2\text{-S-CH}_2\text{-CO-Melle-Leu-Asp-} \\ \text{Val-NH-(CH}_2)_2\text{-S-CH}_2\text{-CO-Melle-Leu-Asp-} \\ \text{Val} \end{array}$	Deltapak column 30-70% (80 min.)
33	$\begin{array}{c} \text{D-Ala-D-Arg(Pbf)-Melle-Leu-Asp(OBut)-Val-} \\ \text{D-Ala-D-Arg(Pbf)-Melle-Leu-Asp(OBut)-Val} \end{array}$	16	$\begin{array}{c} \text{c(D-Ala-D-Arg-Melle-Leu-Asp-Val-D-Ala-} \\ \text{D-Arg-Melle-Leu-Asp-Val} \end{array}$	Deltapak column 20-36% (80 min.)

Preparative HPLC was carried out using a reverse phase (C_{18}) 1 inch diameter Vydac column (218TP1022, 22x250 mm) or a Deltapak (30x300 mm) column. The solvent system consisted of water and acetonitrile (each containing 0.1% trifluoroacetic acid). The column was eluted using a gradient (solvent ratio and time shown in the table) with increasing concentrations of acetonitrile run at a rate of 10 ml/minute. The flow rate on the Deltapak column was 30 ml/min.

Table 2. Characterisation of the Cyclic Dimeric Peptides

Comp. No.	End Product Cyclic Peptide	Amino Acid Analysis (Acid hydrolysis - 6N HCl containing 1% phenol, 24 hours, 130 °C)	HPLC Retention Time (Min.)	Mass Spectroscopy (M+H) ⁺
1		Asp 2.03, Val 1.95, Ile 1.97, Leu 2.01	21.76 10-60% (30 min.)	1133.6
2		Asp 2.05, Val 1.98, Ile 1.98, Leu 2.01	22.46 10-60% (30 min.)	1133.8
3		Asp 2.0, Val 1.96, Ile 1.90, Leu 1.96.	17.5 20-80% (40 min.)	1161.7
4		Asp 1.01, Val 0.98, Ile 0.98, Leu 1.02, β-Ala 0.99	21.23 10-60% (30 min.)	[M+2H] ²⁺ 638.7
5		Asp 2.02, Val 1.98, Ile 1.94, Leu 2.06, β-Ala 0.95	21.43 10-60% (30 min.)	[M+2H] ²⁺ 603.0

6	$\begin{array}{c} \text{OC} - \text{D-Leu-Leu-Asp-Val-NHCH}_2 - \text{CH}_2 \\ \\ \text{CH}_2 - \text{CH}_2 - \text{NH-Val-Asp-Leu-D-Leu} - \text{CO} \end{array}$	Asp 2.10, Val 2.10, Leu 4.0, β -Ala 1.92	19.53 10-80% (30 min.)	1023.7
7	$\begin{array}{c} \text{OC} - \text{Ile-Leu-Asp-Val-N} \\ \quad \quad \quad \\ \text{Cyclohexyl} \quad \quad \quad \text{Cyclohexyl} \\ \quad \quad \quad \\ \text{N-Val-Asp-Leu-Ile} - \text{CO} \end{array}$	Asp 0.98, Val 0.96, Ile 0.96, Leu 1.05	30.2 10-60% (30 min.)	1104
8	c(Melle-Leu-Asp-Val-D-Arg-Melle-Leu-Asp-Val-D-Arg)	Asp 1.02, Val 0.96, Leu 1.02, Arg 1.01.	29.60 20-45% (30 min.)	1221.9 (M+2H) ²⁺ = 611.7
9	c(Melle-Leu-Asp-Val-D-Arg-D-Arg-Melle-Leu-Asp-Val-D-Arg-D-Arg)	Asp 1.03, Val 1.01, Leu 1.03, Arg 1.94.	25.97 10-40% (30 min.)	(M+2H) ²⁺ = 767.8
10	c(Melle-Leu-Asp-Val-D-Arg-D-Arg-Melle-Leu-Asp-Val-D-Ala-D-Ala)	Asp 1.00, Ala 1.02, Val 0.95, Leu 1.01, Arg 1.01.	24.61 20-45% (30 min.)	1363.9 (M+2H) ²⁺ = 682.8
11	$\begin{array}{c} \text{OC} - \text{Melle-Leu-Asp-Val-N} \\ \quad \quad \quad \\ \text{H}_2\text{C-N} \quad \quad \quad \text{N-CH}_2 \\ \quad \quad \quad \\ \text{Cyclohexyl} \quad \quad \quad \text{Cyclohexyl} \\ \quad \quad \quad \\ \text{N-Val-Asp-Leu-Melle} - \text{CO} \end{array}$	Asp 0.98, Val 1.01, Leu 1.01	29.68 10-40% (30 min.)	1161.7

12	$\begin{array}{c} \text{OC-MePhe-Leu-Asp-Val-N} \\ \qquad \qquad \qquad \\ \text{H}_2\text{C-N} \quad \text{N-Val-Asp-Leu-MePhe CO} \\ \qquad \qquad \qquad \\ \text{CH}_2 \quad \text{CH}_2 \end{array}$	Asp 1.02, Val 0.96, Leu 1.02	28.27 10-60% (30 min.)	1229.9 (M+2H) ²⁺ = 615.7
13	$\begin{array}{c} \text{OC-D-Arg-MePhe-Leu-Asp-Val-N} \\ \qquad \qquad \qquad \\ \text{H}_2\text{C-N} \quad \text{N-Val-Asp-Leu-MePhe-D-Arg-CO} \\ \qquad \qquad \qquad \\ \text{CH}_2 \quad \text{CH}_2 \end{array}$	Asp 1.03, Val 0.96, Leu 1.02, Arg 0.99.	24.01 10-60% (30 min.)	(M+2H) ²⁺ = 771.8
14	$\begin{array}{c} \text{OC-Melle-Leu-Asp-Val-N} \\ \qquad \qquad \qquad \\ \text{H}_2\text{C-N} \quad \text{N-Val-Asp-Leu-Melle-CO} \\ \qquad \qquad \qquad \\ \text{CH}_2 \quad \text{CH}_2 \end{array}$	Asp 1.02, Val 0.95, Leu 1.03.	25.54 20-45% (30 min.)	(M+2H) ²⁺ = 595.8
15	c(NH-(CH ₂) ₂ -S-CH ₂ -CO-Melle-Leu-Asp-Val- NH-(CH ₂) ₂ -S-CH ₂ -CO-Melle-Leu-Asp-Val)	Asp 1.03, Val 0.96, Leu 1.02.	21.53 30-70% (30 min.)	1143.7
16	c(D-Ala-D-Arg-Melle-Leu-Asp-Val-D-Ala-D- Arg-Melle-Leu-Asp-Val)	Asp 1.02, Ala 1.03, Val 0.97, Leu 1.0, Arg 1.02.	25.38 20-45% (30 min.)	1364.1 (M+2H) ²⁺ = 682.5

Analytical HPLC was carried out using either a reverse phase (C₁₈) Vydac column (218TP54, 4.6x250 mm) or a Novapak column (3.9x150 mm). Unless otherwise stated in the above table a Vydac column was used for the compound. The solvent system consisted of water and acetonitrile (each containing 0.1% trifluoroacetic acid). The column was eluted using a gradient (solvent ratio and time shown in the table) with increasing concentrations of acetonitrile run at a rate of 1 ml/minute.

The following data gives the sequence listing generated using the patentin software which has to be provided for some patent offices.

All compounds in the following listing are represented diagrammatically in Tables 1 and 2.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: ZENECA LIMITED
(B) STREET: 15 STANHOPE GATE
(C) CITY: LONDON
(E) COUNTRY: UNITED KINGDOM
(F) POSTAL CODE (ZIP): W1Y 6LN
(G) TELEPHONE: 0171 304 5000
(H) TELEFAX: 0171 304 5151
(I) TELEX: 0171 834 2042

(ii) TITLE OF INVENTION: CHEMICAL COMPOUNDS

(iii) NUMBER OF SEQUENCES: 33

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Peptide
(B) LOCATION:5
(D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(ix) FEATURE:

(A) NAME/KEY: Peptide
(B) LOCATION:10
(D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Ile Leu Asp Val Xaa Ile Leu Asp Val Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: circular

- 39 -

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:1
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-LEU"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:5
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:6
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-LEU"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:10
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Xaa	Leu	Asp	Val	Xaa	Xaa	Leu	Asp	Val	Xaa
1				5					10

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:5
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-PROPIONYL"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:10
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-PROPIONYL"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Ile	Leu	Asp	Val	Xaa	Ile	Leu	Asp	Val	Xaa
1				5					10

- 40 -

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:1
- (D) OTHER INFORMATION:/product= "bAla"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:6
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:7
- (D) OTHER INFORMATION:/product= "bAla"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:12
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ala Ile Leu Asp Val Xaa Ala Ile Leu Asp Val Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:1
- (D) OTHER INFORMATION:/product= "bAla"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:6
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(ix) **FEATURE:**

- (A) NAME/KEY: Peptide
(B) LOCATION:11
(D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ala Ile Leu Asp Val Xaa Ile Leu Asp Val Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
(B) LOCATION:1
(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Leu"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
(B) LOCATION:5
(D) OTHER INFORMATION: /product= "OTHER"
/note= "-NH-CH2-CH2-CO-"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
(B) LOCATION: 6
(D) OTHER INFORMATION: /product= "OTHER"
/note= "D-LEU"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
(B) LOCATION:10
(D) OTHER INFORMATION:/product= "OTHER"
/note= "-NH-CH2-CH2-CO-"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Xaa Leu Asp Val Xaa Xaa Leu Asp Val Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

- 42 -

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:5
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERIDYL-5-ACETYL"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:10
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERIDYL-5-ACETYL"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Ile	Leu	Asp	Val	Xaa	Ile	Leu	Asp	Val	Xaa
1			5						10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:1
- (D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:5
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:6
- (D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:10
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Ile	Leu	Asp	Val	Xaa	Ile	Leu	Asp	Val	Xaa
1			5						10

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) NAME/KEY: Peptide
(B) LOCATION:1
(D) OTHER INFORMATION:/product= "Melle"

(A) NAME/KEY: Peptide
(B) LOCATION:5
(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg"

(A) NAME/KEY: Peptide
(B) LOCATION:6
(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg"

(A) NAME/KEY: Peptide
(B) LOCATION:7
(D) OTHER INFORMATION:/product= "Melle"

(A) NAME/KEY: Peptide
(B) LOCATION:11
(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg"

(A) NAME/KEY: Peptide
(B) LOCATION:12
(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg"

Ile Leu Asp Val Xaa Xaa Ile Leu Asp Val Xaa Xaa
1 5 10

(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: circular

(A) NAME/KEY: Peptide
(B) LOCATION:1

- 44 -

(D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:5

(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:6

(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:7

(D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:11

(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Ala"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:12

(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Ala"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Ile	Leu	Asp	Val	Xaa	Xaa	Ile	Leu	Asp	Val	Xaa	Xaa
1				5						10	

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:1

(D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:5

(D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:6

- 45 -

(D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:10

(D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Ile	Leu	Asp	Val	Xaa	Ile	Leu	Asp	Val	Xaa
1				5					10

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:1

(D) OTHER INFORMATION:/product= "OTHER"
/note= "MePhe"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:5

(D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:6

(D) OTHER INFORMATION:/product= "OTHER"
/note= "MePhe"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:10

(D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Xaa	Leu	Asp	Val	Xaa	Xaa	Leu	Asp	Val	Xaa
1				5					10

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:5
- (D) OTHER INFORMATION:/product= "OTHER" ...
/note= "1,4-DIAZEPAN-1-YL-ACETYL"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:6
- (D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:10
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "1,4-DIAZEPAN-1-YL-ACETYL"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Ile	Leu	Asp	Val	Xaa	Ile	Leu	Asp	Val	Xaa
1				5					10

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:1
- (D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:5
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "-NH-(CH2)2-S-CH2-CO-"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:6
- (D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:10
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "-NH-(CH2)2-S-CH2-CO-"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Ile	Leu	Asp	Val	Xaa	Ile	Leu	Asp	Val	Xaa
1				5					10

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
(B) LOCATION:1
(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Ala"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
(B) LOCATION:2
(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg" - -

(ix) **FEATURE:**

- (A) NAME/KEY: Peptide
(B) LOCATION:3
(D) OTHER INFORMATION:/product= "Melle"

(ix) **FEATURE:**

- (A) NAME/KEY: Peptide
(B) LOCATION: 7
(D) OTHER INFORMATION: /product= "OTHER"
/note= "D-Ala"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
(B) LOCATION:8
(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
(B) LOCATION: 9
(D) OTHER INFORMATION: /product= "Melle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Xaa Xaa Ile Leu Asp Val Xaa Xaa Ile Leu Asp Val
1 5 10

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:3
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:5
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETIC ACID"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Ile Leu Xaa Val Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:3
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:5
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:8
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:10
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETIC ACID"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Ile Leu Xaa Val Xaa Ile Leu Xaa Val Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO: 19:

- 50 -

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:1
(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Leu"

- (ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:3
(D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

- (ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:5
(D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETIC ACID"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Xaa Leu Xaa Val Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 20:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:3
(D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

- (ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:5
(D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-PROPIONYL"

- (ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:8
(D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

- 51 -

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:10
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-PROPIONIC ACID"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Ile Leu Xaa Val Xaa Ile Leu Xaa Val Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:1
- (D) OTHER INFORMATION:/product= "bAla"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:4
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut) "

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:6
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:7
- (D) OTHER INFORMATION:/product= "bAla"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:10
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut) "

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:12
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETIC ACID"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Ala Ile Leu Xaa Val Xaa Ala Ile Leu Xaa Val Xaa
1 5 10

- 52 -

(2) INFORMATION FOR SEQ ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 11 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:
- (A) NAME/KEY: Peptide
 - (B) LOCATION:1
 - (D) OTHER INFORMATION:/product= "bAla"

- (ix) FEATURE:
- (A) NAME/KEY: Peptide
 - (B) LOCATION:4
 - (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

- (ix) FEATURE:
- (A) NAME/KEY: Peptide
 - (B) LOCATION:6
 - (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1YL-ACETYL"

- (ix) FEATURE:
- (A) NAME/KEY: Peptide
 - (B) LOCATION:9
 - (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

- (ix) FEATURE:
- (A) NAME/KEY: Peptide
 - (B) LOCATION:11
 - (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETIC ACID"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Ala Ile Leu Xaa Val Xaa Ile Leu Xaa Val Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO: 23:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:
- (A) NAME/KEY: Peptide
 - (B) LOCATION:1
 - (D) OTHER INFORMATION:/product= "OTHER"

- 53 -

/note= "D-Leu"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:5
- (D) OTHER INFORMATION:/product= "bAla"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:6
- (D) OTHER INFORMATION:/product= "OTHER"
- /note= "D-Leu"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:10
- (D) OTHER INFORMATION:/product= "bAla"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Xaa Leu Asp Val Ala Xaa Leu Asp Val Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:1
- (D) OTHER INFORMATION:/product= "OTHER"
- /note= "Asp(OBut)"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:3
- (D) OTHER INFORMATION:/product= "OTHER"
- /note= "PIPERIDYL-5-ACETYL"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Xaa Val Xaa Ile Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- 54 -

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:1
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg(Pbf) where Pbf is
2,2,4,6,7-pentamethyl-dihydrobenzenefuran-5-sulphonyl"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:2
- (D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:4
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:6
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg(Pfb) where Pfb is
2,2,4,6,7-pentamethyldihydrobenzenefuran-5-sulphonyl"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:7
- (D) OTHER INFORMATION:/product= "Melle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Xaa	Ile	Leu	Xaa	Val	Xaa	Ile	Leu	Asp	Val
1			5					10	

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:1
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg(Pfb) Where Pfb is
2,2,4,6,7-pentamethyldihydrobenzenefuran-5-sulphonyl"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:2
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg(Pfb) where Pfb is
2,2,4,6,7-pentamethyldihydrobenzenefuran-5-sulphonyl"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:3
- (D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:5
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:7
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg(Pfb) where Pfb is
2,2,4,6,7,-pentamethyldihydrobenzenefuran-5-sulphonyl"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:8
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg(Pfb) where Pfb is
2,2,4,6,7,-pentamethyldihydrobenzenefuran-5-sulphonyl"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:9
- (D) OTHER INFORMATION:/product= "Melle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Xaa	Xaa	Ile	Leu	Xaa	Val	Xaa	Xaa	Ile	Leu	Asp	Val
1			5						10		

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:1
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Ala"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:2
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Ala"

(ix) FEATURE:

- (A) NAME/KEY: Peptide

- 56 -

(B) LOCATION:3

(D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:5

(D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:7

(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg(Pfb) where Pfb is
2,2,4,6,7-pentamethyldihydrobenzenefuran-5-sulphonyl"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:8

(D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg(Pfb) where Pfb is
2,2,4,6,7-pentamethyldihydrobenzenefuran-5-sulphonyl"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:9

(D) OTHER INFORMATION:/product= "Melle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Xaa	Xaa	Ile	Leu	Xaa	Val	Xaa	Xaa	Ile	Leu	Asp	Val
1				5					10		

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:1

(D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:3

(D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:5

(D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

```
(ix) FEATURE:
      (A) NAME/KEY: Peptide
      (B) LOCATION:6
      (D) OTHER INFORMATION:/product= "Melle"
```

(ix) FEATURE:
 (A) NAME/KEY: Peptide
 (B) LOCATION: 8
 (D) OTHER INFORMATION: /product= "OTHER"
 /note= "Asp(OBut)"

```
(ix) FEATURE:
      (A) NAME/KEY: Peptide
      (B) LOCATION:10
      (D) OTHER INFORMATION:/product= "OTHER"
           /note= "PIPERAZINYL-1-YL-ACETIC ACID"
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Ile Leu Xaa Val Xaa Ile Leu Xaa Val Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
 (A) NAME/KEY: Peptide
 (B) LOCATION:1
 (D) OTHER INFORMATION:/product= "OTHER"
 /note= "MePhe"

```
(1x) FEATURE:
      (A) NAME/KEY: Peptide
      (B) LOCATION:3
      (D) OTHER INFORMATION:/product= "OTHER"
           /note= "Asp(OBut)"
```

```
(ix) FEATURE:
      (A) NAME/KEY: Peptide
      (B) LOCATION:5
      (D) OTHER INFORMATION:/product= "OTHER"
           /note= "PIPERAZINYLYL-1-YL-ACETYL"
```

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:6
(D) OTHER INFORMATION:/product= "OTHER"
/note= "MePhe"

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:8
(D) OTHER INFORMATION:/product= "OTHER"

- 58 -

/note= "Asp(OBut)"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:10
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETIC ACID"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Xaa	Leu	Xaa	Val	Xaa	Xaa	Leu	Xaa	Val	Xaa
1				5					10

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:1
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg(Pfb) where Pfb is
2,2,4,6,7-pentamethyldihydrobenzenefuran-5-sulphonyl"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:2
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "MePhe"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:4
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:6
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETYL"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:7
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg(Pfb) where Pfb is
2,2,4,6,7-pentamethyldihydrobenzenefuran-5-sulphonyl"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:8
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "MePhe"

- 59 -

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:10
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:12
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "PIPERAZINYL-1-YL-ACETIC ACID"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Xaa Xaa Leu Xaa Val Xaa Xaa Xaa Leu Xaa Val Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:1
- (D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:3
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:5
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "1,4,-DIAZEPAN-1-YL-ACETYL"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:6
- (D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:8
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:10
- (D) OTHER INFORMATION:/product= "OTHER"

- 60 -

/note= "1,4-DIAZEPAN-1-YL-ACETIC ACID"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Ile	Leu	Xaa	Val	Xaa	Ile	Leu	Xaa	Val	Xaa
1				5					10

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:1
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "NH₂-(CH₂)₂-S-CH₂-CO-MeIle"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:3
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:5
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "NH-(CH₂)₂-S-CH₂-CO-"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:6
- (D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:8
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Xaa	Leu	Xaa	Val	Xaa	Ile	Leu	Xaa	Val
1				5				

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- 61 -

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:1
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Ala"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:2
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg(Pfb) where Pfb is
2,2,4,6,7-pentamethyldihydrobenzenefuran-5-sulphonyl"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:3
- (D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:5
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:7
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Ala"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:8
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "D-Arg(Pfb) where Pfb is
2,2,4,6,7-pentamethyldihydrobenzenefuran-5-sulphonyl"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION:9
- (D) OTHER INFORMATION:/product= "Melle"

(ix) FEATURE:

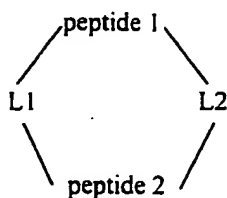
- (A) NAME/KEY: Peptide
- (B) LOCATION:11
- (D) OTHER INFORMATION:/product= "OTHER"
/note= "Asp(OBut)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Xaa Xaa Ile Leu Xaa Val Xaa Xaa Ile Leu Xaa Val
1 5 10

CLAIMS

1. A cyclic dimeric peptide of formula 1



Formula 1

5

wherein peptide 1 and peptide 2 independently represent a tetrapeptide of formula
-AA1-AA2-AA3-AA4- juxtaposed in parallel or antiparallel orientation;

and

AA1 is an L or D amino acid selected from Ile, Leu and amino analogues thereof selected
10 from Pro, Gly, Tic, Gln, and Phe;

AA2 is an L amino acid selected from Leu and amino acid analogues thereof selected from
Ile, Phe, Pro and Val;

AA3 is an L amino acid selected from Asp, Glu and amino acid analogues thereof;

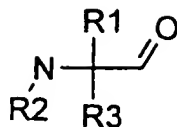
AA4 is an L amino acid selected from Val and amino acid analogues thereof selected from
15 Leu, Ile, Phe, Ser, Pro and Cha (cyclohexylalanine);

L1 and L2 independently represent linking moieties for linking peptides 1 and 2 to form a
cyclic peptide; or a salt thereof.

2. A cyclic dipeptide according to claim 1 wherein

20 AA1 to AA4 each have the general formula 2

- 63 -



Formula 2

wherein R^1 is the amino acid side chain and R^2 and R^3 are independently selected from H and C_{1-4} alkyl; or a salt thereof.

5

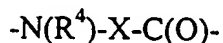
3. A cyclic dipeptide according to claim wherein

AA1 is selected from Ile, D-Leu, Melle and MePhe; AA2 is Leu; AA3 is Asp; and AA4 is Val, or a salt thereof.

10 4. A cyclic dipeptide according to any one of claims 1 to 3 wherein the peptides 1 and 2 are in antiparallel juxtaposition or a salt thereof.

5. A cyclic dipeptide according to claim 4 wherein L1 and L2 are independently selected from a group of formula

15



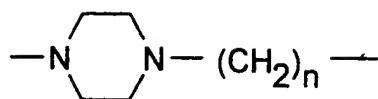
wherein:-

R^4 is selected from H and C_{1-4} alkyl, X represents $-(\text{CH}_2)_n-$ wherein $n = 1-4$, optionally

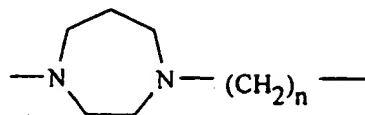
20 substituted on $-\text{CH}_2$ with C_{1-4} alkyl or NH_2 ; or

X and NR^4 together represent a group of

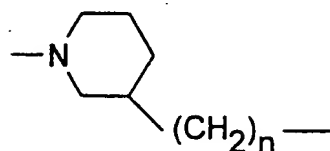
- 64 -



5



10



in which $n = 0-4$; or a salt thereof.

15 6. A cyclic dipeptide according to claim 4 wherein L1 and L2 are independently selected from any one of the formulae as set out in figure 7 herein or a salt thereof.

7. A cyclic dipeptide according to claim 4 wherein L1 and L2 are independently selected from any one of formulae 1 to 12 as set out in figure 17 herein or a salt thereof.

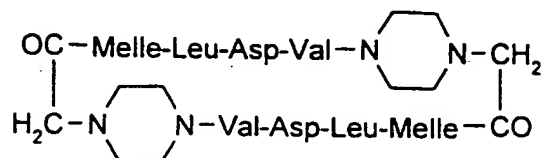
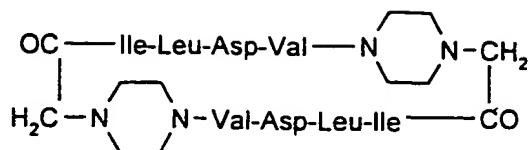
20

8. A cyclic dipeptide according to claim 7 wherein L1 and L2 are independently selected from any one of formulae 1, 9 and 10 to 12 as set out in figure 17 herein or a salt thereof.

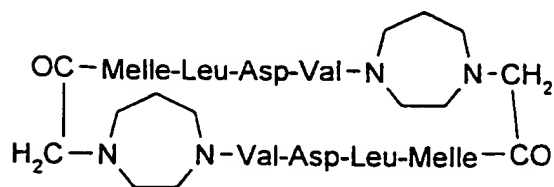
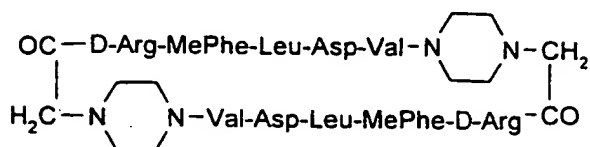
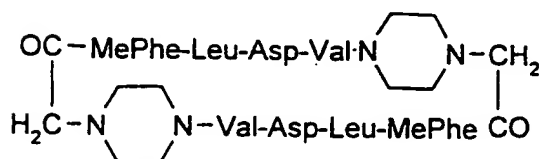
25 9. A cyclic dipeptide according to any one of claims 1 to 8 in which L1 and L2 are identical.

10. A cyclic dipeptide selected from

- 65 -



5



10

or a salt thereof.

11. A process for the manufacture of a cyclic dipeptide of formula 1 selected from:-

- 15 (a) removing one or more conventional peptide protecting groups from a protected polypeptide of formula 10 as set out in figure 10 herein, wherein Pr1 is a protecting group on the acid group in the side chain of AA3, to give a cyclic peptide polypeptide of the invention of formula 1 and, optionally, simultaneously or subsequently, also removing any

- 66 -

additional conventional peptide protecting groups and optionally converting the product thus obtained into a salt thereof; and

- (b) forming an amide bond by coupling two peptide units, one containing a carboxylic acid group, or a reactive derivative thereof, and the other containing an amino group, such that a protected or unprotected cyclic peptide having the sequence indicated in formula 1 is produced and, if necessary, removing any protecting groups using process (a) above and optionally converting the product thus obtained into a salt thereof.

12. A pharmaceutical composition comprising a cyclic dipeptide according to any one of claims 1 to 9 or a salt thereof in association with a pharmaceutically acceptable diluent or carrier.

13. A pharmaceutical composition according to claim 12 for parenteral administration designed for slow release over a period of at least five days.

14. A cyclic peptide according to any one of claims 1 to 10 for use as a medicament.

15. A method for inhibiting the interaction between VCAM-1 and/or fibronectin and the integrin receptor VLA-4 in mammals in need of such treatment comprising administering to said mammals an effective amount of a cyclic dipeptide according to any one of claims 1 to 9 or a pharmaceutically acceptable salt thereof.

16. A method according to claim 15 wherein the mammal in need of treatment is suffering from multiple sclerosis, asthma or rheumatoid arthritis.

17. Use of a cyclic dipeptide of formula 1 or a pharmaceutically-acceptable salt thereof in the production of a medicament for use in the treatment of a disease or medical condition mediated by the interaction between VCAM-1 and the integrin receptor VLA-4.

30

Fig.1.

Formula 1

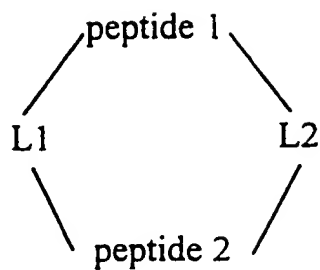


Fig.2.

Formula 2

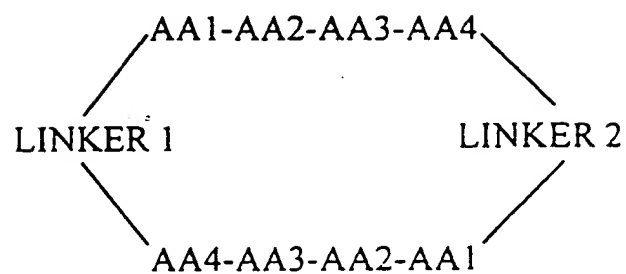


Fig.3.

Formula 3

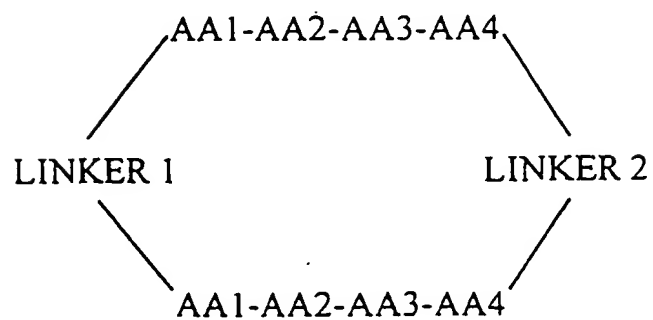
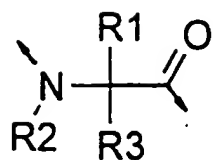
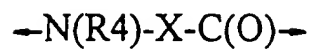


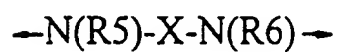
Fig.4.



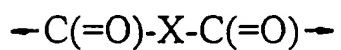
Formula 4



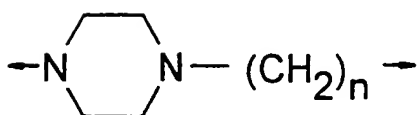
Formula 5



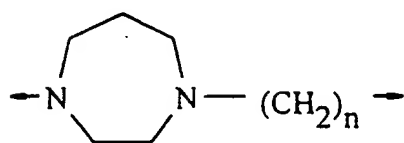
Formula 6



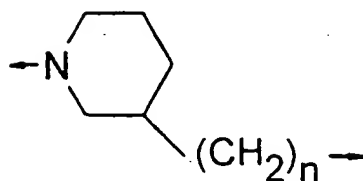
Formula 7



Formula 8



Formula 8a

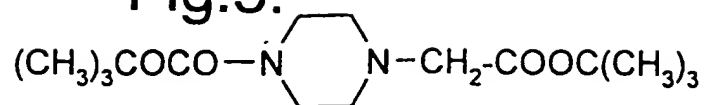


Formula 9

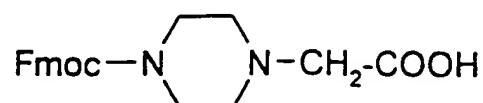
3/11

Fig.5.

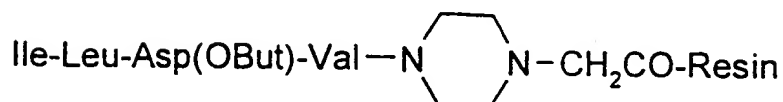
Step 1



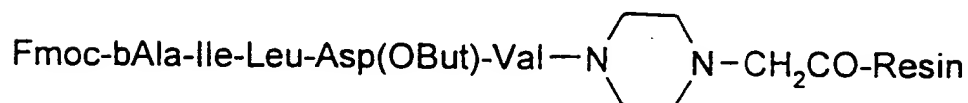
Step 2



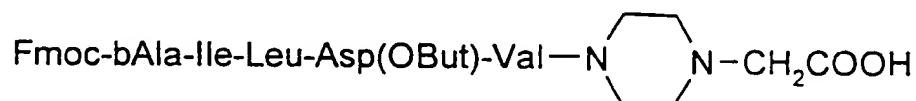
Step 3



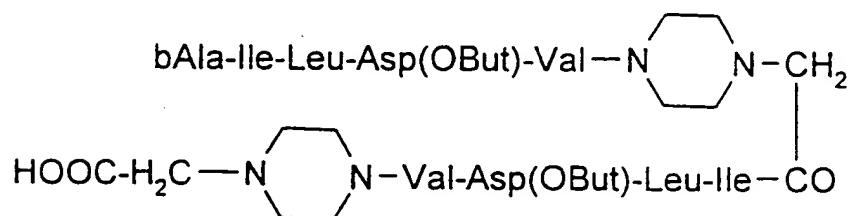
Step 4



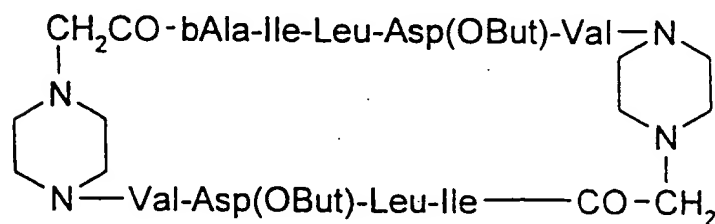
Step 5



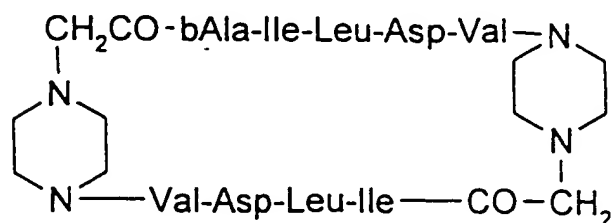
Step 6



Step 7



Step 8



4/11

Step 1

Fig.6.

Fmoc—Val-Chlorotritylresin



Step 2.

Asp(OBut)-Val-D-Arg(Pbf)-Melle-Leu-Asp(OBut)-Val-Chlorotritylresin
 |
 Leu-Melle-(Pbf)Arg-D-Fmoc



Piperidine

Step 3

Asp(OBut)-Val-D-Arg(Pbf)-Melle-Leu-Asp(OBut)-Val-Chlorotritylresin
 |
 Leu-Melle-(Pbf)Arg-D



Acetic acid/Trifluoroethanol
 Dichloromethane

Step 4.

D-Arg(Pbf)-Melle-Leu—Asp(OBut)-Val-D-Arg(Pbf)-Melle-Leu-Asp(OBut)-Val



Cyclisation

Step 5

c(D-Arg(Pbf)-Melle-Leu—Asp(OBut)-Val-D-Arg(Pbf)-Melle-Leu-Asp(OBut)-Val)

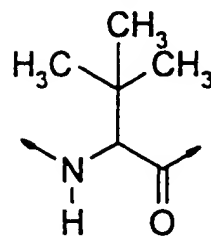


Trifluoroacetic acid/water/
 Triisopropylsilane

Step 6.

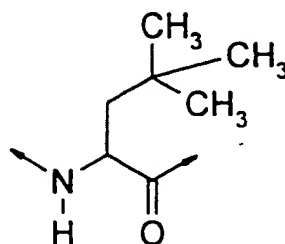
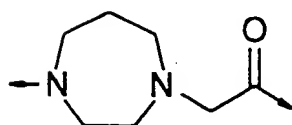
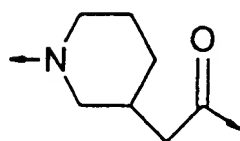
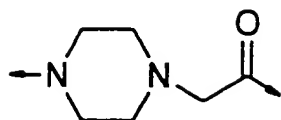
c(D-Arg-Melle-Leu—Asp-Val-D-Arg-Melle—Leu-Asp-Val)

Fig.8.



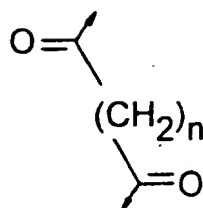
[t-butyl-glycine, t-leucine]

Fig.7.

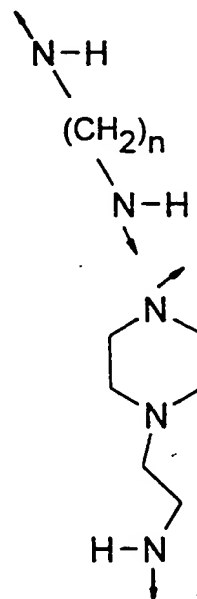
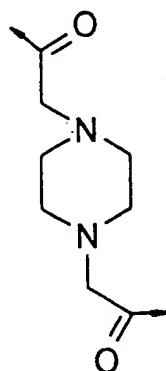


[t-butyl-alanine, neopentylglycine]

Fig.12.

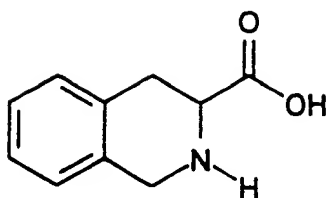


n=1-4



6/11

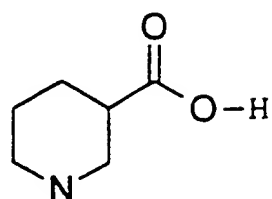
Fig.9.



1

Fig.10.

Formula 10



2

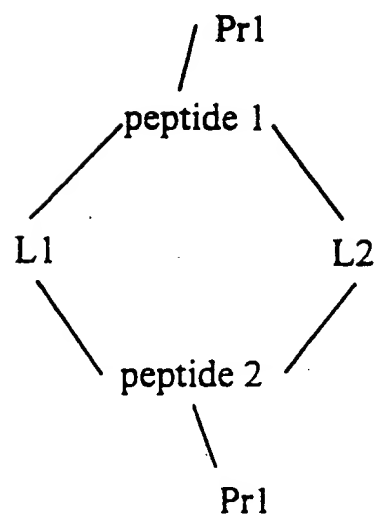
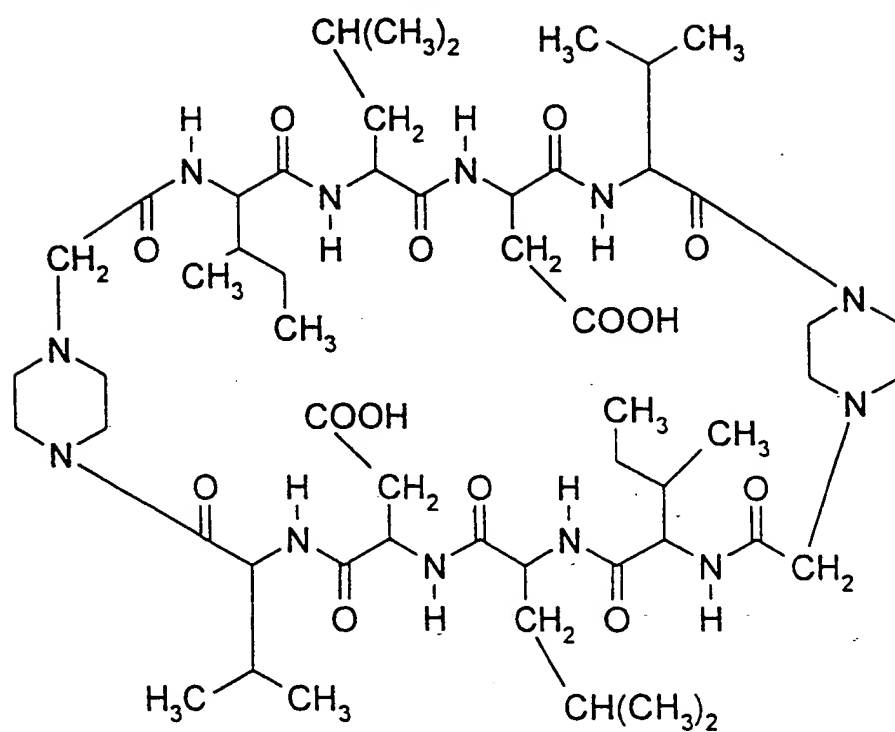
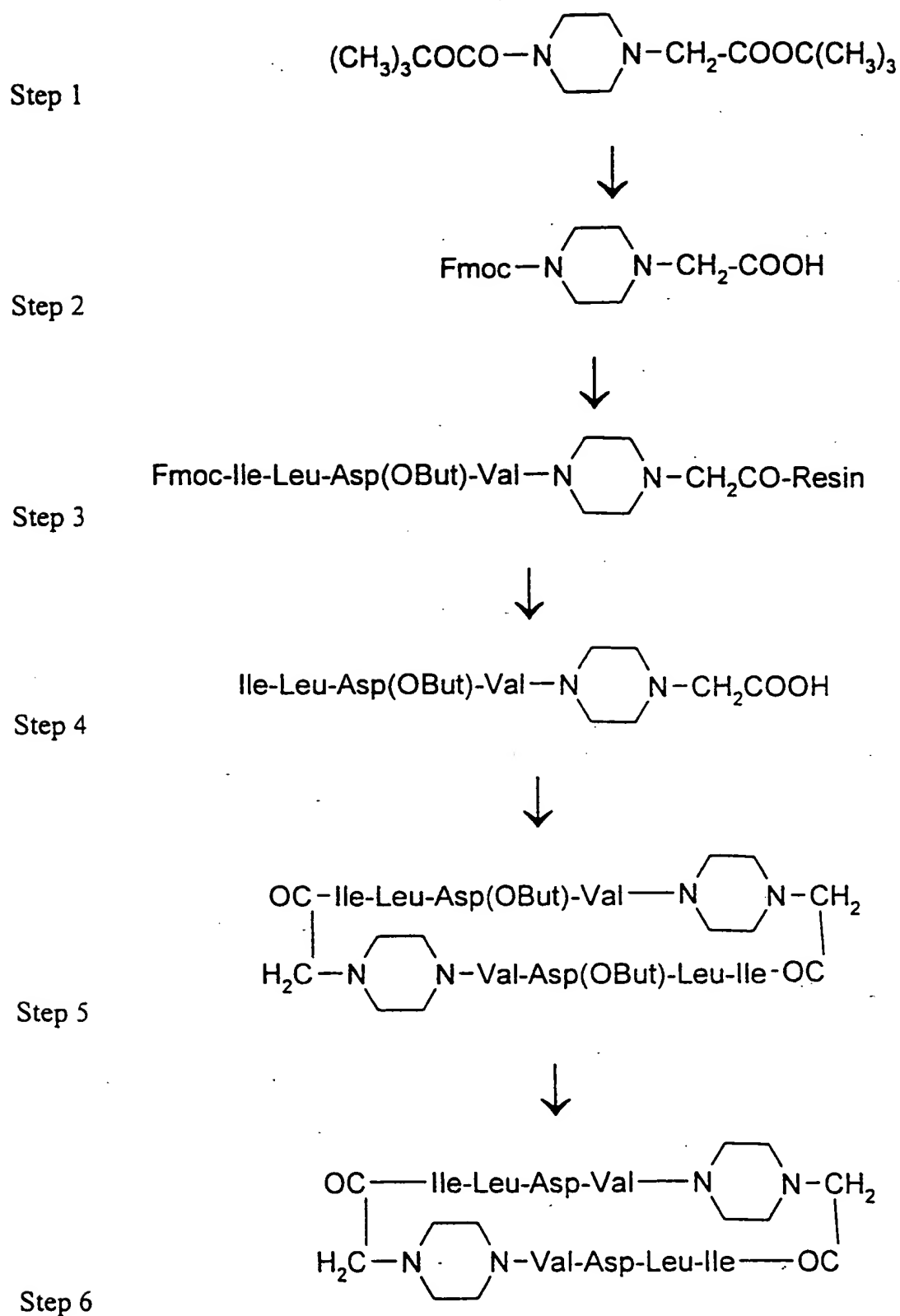


Fig.11.



7/11

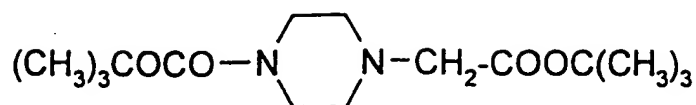
Fig.13.



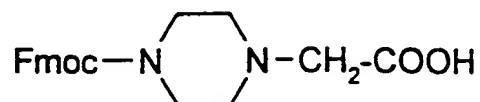
8/11

Fig.14.

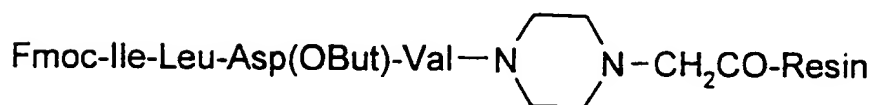
Step 1



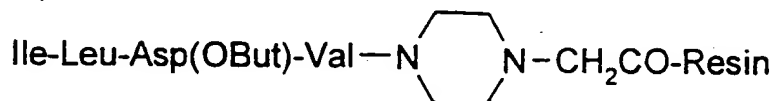
Step 2



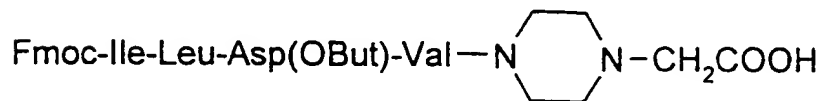
Step 3



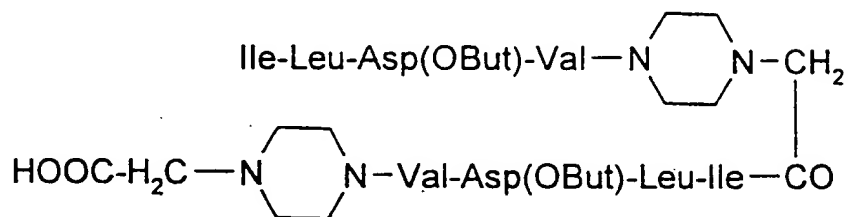
Step 4



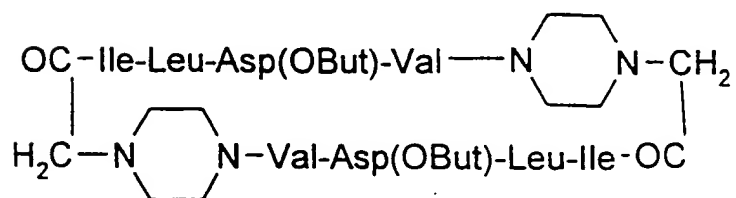
Step 5



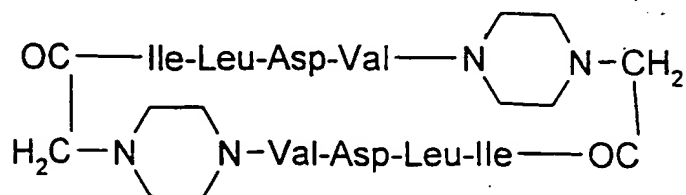
Step 6



Step 7



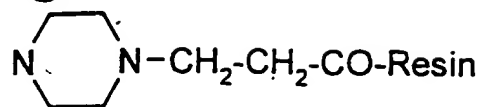
Step 8



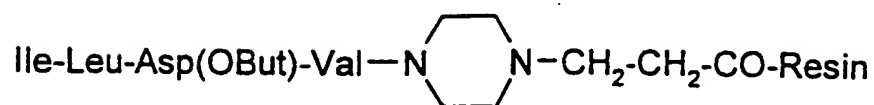
9/11

Fig.15.

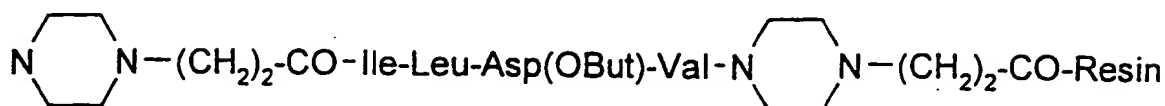
Step 1



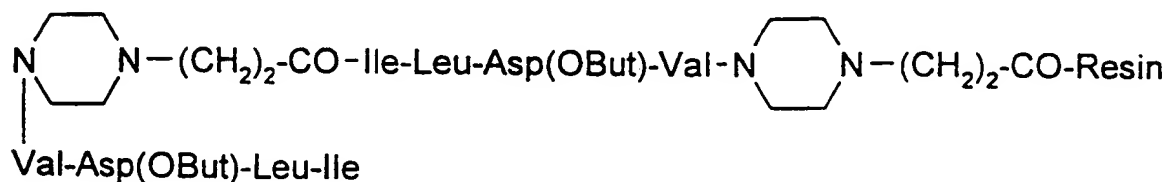
Step 2



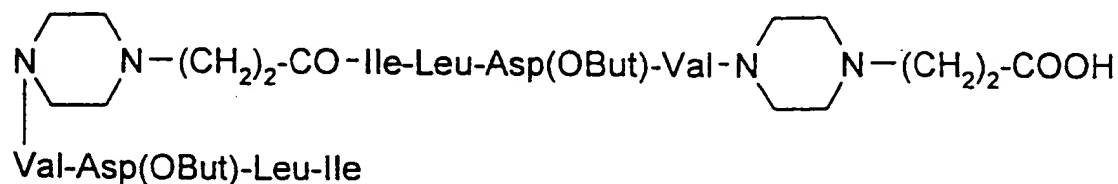
Step 3



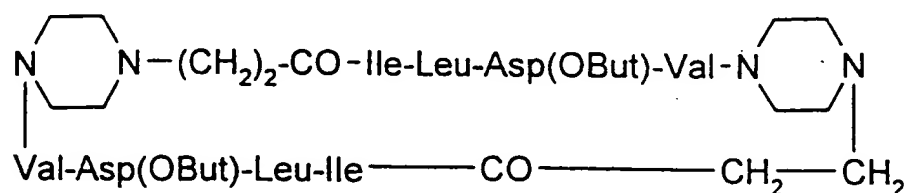
Step 4



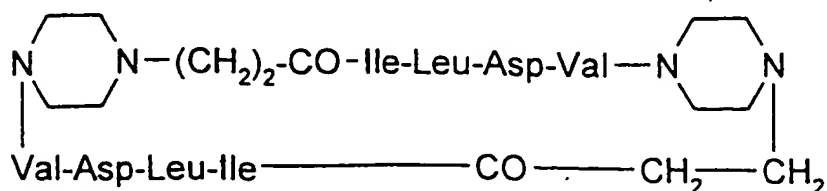
Step 5



Step 6



Step 7

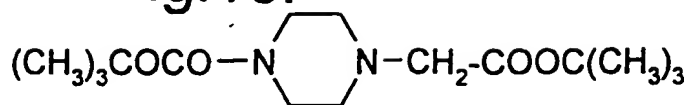


SUBSTITUTE SHEET (RULE 26)

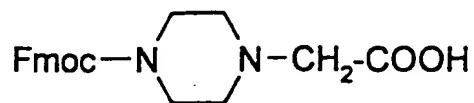
10/11

Fig.16.

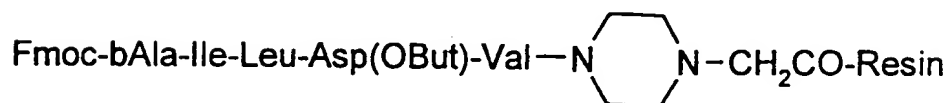
Step 1



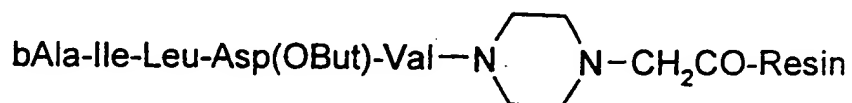
Step 2



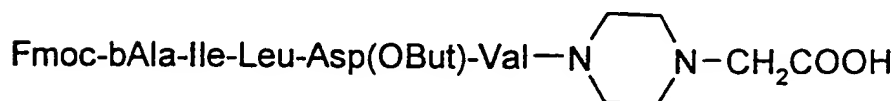
Step 3



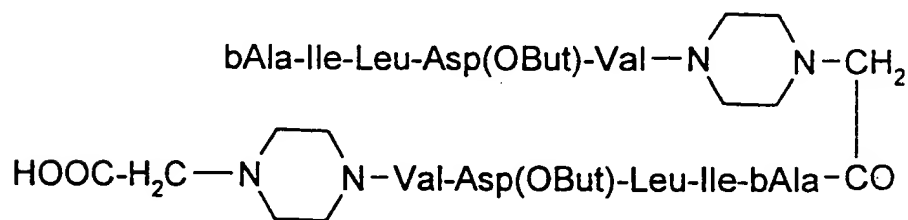
Step 4



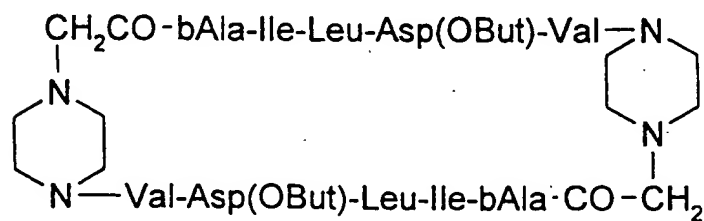
Step 5



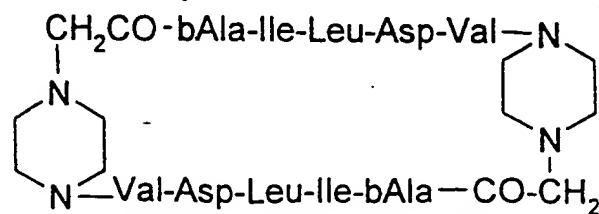
Step 6



Step 7



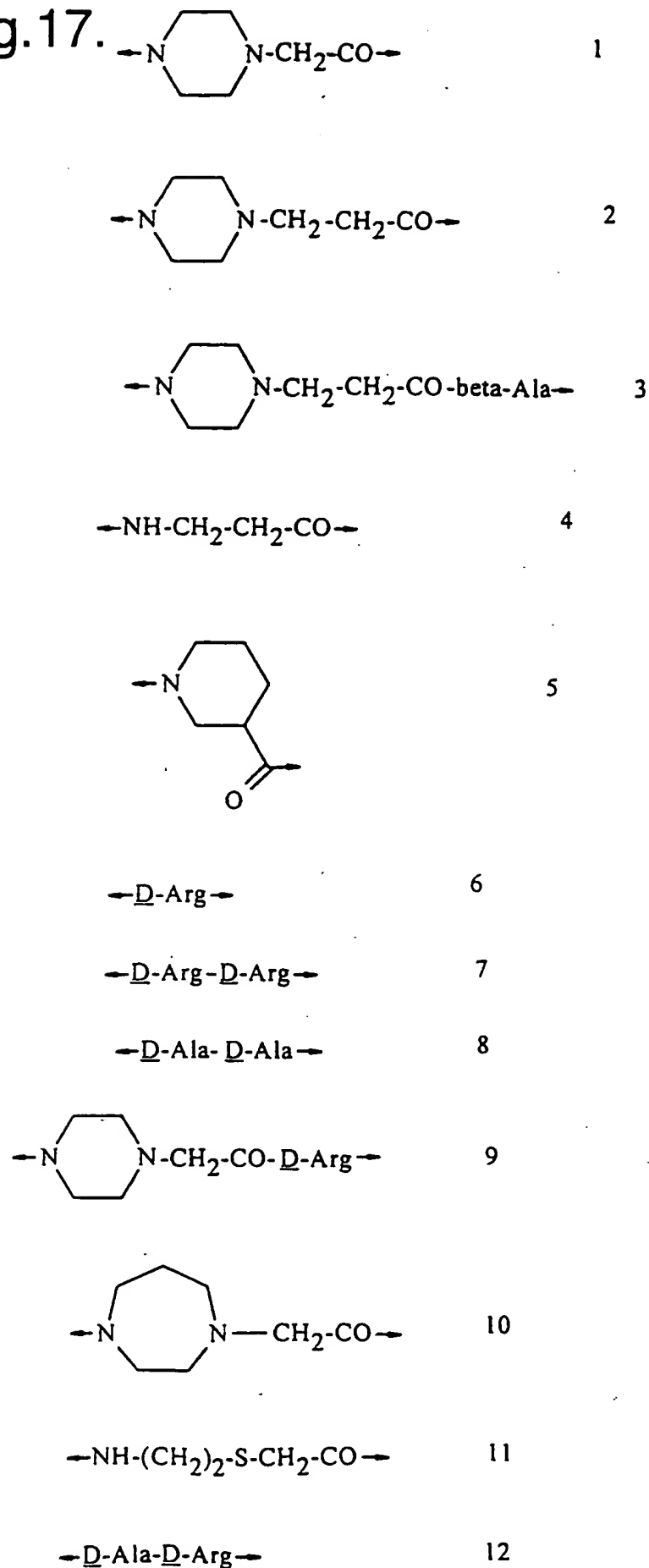
Step 8



SUBSTITUTE SHEET (RULE 26)

11/11

Fig.17.



INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 96/01580

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/78, C07K 7/56, A61K 38/39, A61K 38/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

REG, CAPLUS, WPI, IFIPAT, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO, A1, 9620216 (ZENECA LIMITED), 4 July 1996 (04.07.96) --	1-14,17
P,X	WO, A1, 9600581 (TEXAS BIOTECHNOLOGY CORPORATION), 11 January 1996 (11.01.96) --	1-14,17
X	WO, A1, 9515973 (CYTEL CORPORATION), 15 June 1995 (15.06.95) --	1-14,17
A	US, A, 5229366 (YOSHIHISA TSUKADA ET AL A), 20 July 1993 (20.07.93) --	1-14,17

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

30 Sept. 1996

Date of mailing of the international search report

22.10.96

Name and mailing address of the ISA/



European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

CAROLINA GÓMEZ LAGERLÖF

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 96/01580

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO, A2, 9415958 (TANABE SEIYAKU CO., LTD.), (21.07.04)</p> <p style="text-align: center;">-- -----</p>	1-14, 17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB-96/01580

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 15, 16
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(i.v.) Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.
2. ☒ Claims Nos.: 1
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See enclosure
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

05/09/96

International application No.

PCT/GB 96/01580

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9620216	04/07/96	GB-D- 9426254 GB-D- 9524630 GB-D- 9505905 GB-D- 9513904	00/00/00 00/00/00 00/00/00 00/00/00
WO-A1- 9600581	11/01/96	AU-A- 2958195	25/01/96
WO-A1- 9515973	15/06/95	AU-A- 1215695 CA-A- 2177840	27/06/95 15/06/95
US-A- 5229366	20/07/93	JP-A- 4217693 JP-A- 4187698	07/08/92 06/07/92
WO-A2- 9415958	21/07/04	CA-A- 2153228 EP-A- 0677060 JP-T- 8505628	21/07/94 18/10/95 18/06/96

THIS PAGE BLANK (USPTO)